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Effects of various Ca^{2+} channel antagonists on morphine analgesia, tolerance and dependence, and on blood pressure in the rat

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Abstract

Following the finding that nifedipine enhances morphine analgesia and prevents the development of dependence, we have now compared the effect of nifedipine with these of other L-type Ca^{2+} channel antagonists, nimodipine (a dihydropyridine) and verapamil (a phenylethylalkylamine). Male Wistar rats received the antagonist 20 min before each injection of morphine. Analgesia was measured in a hot-plate test, and the development of dependence was assessed in the naloxone precipitation test after 13 days of morphine (20–30 mg/kg i.p.) administration. L-type Ca^{2+} channels were assayed in the cerebral cortex as [^{3}H]nitrendipine binding sites. Blood pressure was monitored from the tail by a non-invasive method. We found that all three Ca^{2+} antagonists enhanced the analgesia, and prevented development of the naloxone-precipitated withdrawal syndrome, although they differed in their efficacy. Nifedipine and verapamil effectively blocked the development of tolerance. While chronic morphine up-regulated L-type Ca^{2+} channels, co-administration of the antagonists completely prevented this effect. The effects of Ca^{2+} channel antagonists cannot be ascribed to their potential circulatory effects as, at the dose used, none affected significantly the arterial blood pressure.

Keywords: Ca^{2+} channel antagonist; Morphine; Analgesia; Tolerance dependence; Blood pressure; [^{3}H]Nitrendipine binding site

1. Introduction

Ca^{2+} channel antagonists were found by us and by others to modify the action of morphine in a manner favorable from the clinical point of view. Thus, Ca^{2+} channel antagonists were reported to enhance morphine analgesia and to prevent the naloxone-induced morphine abstinence syndrome (Antkiewicz-Michaluk et al., 1990, 1993; Hoffmeister and Tettenborn, 1986; Kavaliers, 1987; Contreras et al., 1988; Hodoglugil et al., 1996; Omote et al., 1993). Moreover, we found that co-administration of a dihydropyridine Ca^{2+} channel antagonist, nifedipine, together with morphine in a chronic experiment, alleviates the tolerance and prevents the development of dependence, as shown by reduction of the ability of naloxone to precipitate the behavioral and biochemical signs of the abstinence syndrome (Antkiewicz-Michaluk et al., 1993).

As far as application of these findings to the clinic is concerned, in addition to the warning that nifedipine may cause confusion in morphine-dependent patients during the withdrawal syndrome (Silverstone et al., 1992), objections could be raised that Ca^{2+} channel antagonists are known hypotensive agents and, therefore, their action may produce unwanted side-effects. In fact, it was reported that some interactions between morphine and nifedipine were similar to those between morphine and hydralazine, a non-Ca^{2+} channel antagonist vasodilator (Pavone et al., 1992). Although earlier reports indicate that Ca^{2+} channel antagonists, while effective hypotensive agents in hypertensive rats, only slightly, if at all, depress the blood pressure of normotensive animals (Ueda et al., 1993; Sharma et al., 1984; Ishii et al., 1980), data concerning the effects of their chronic administration are lacking.

We now extended our previous studies with nifedipine to other L-type Ca^{2+} channel antagonists, which were investigated both for their modulatory action on morphine analgesia, tolerance and dependence, and for their effects on blood pressure of rats receiving them acutely or chronically. The compounds studied were two dihydropyridine Ca^{2+} channel antagonists, nifedipine, regarded as exerting a predominantly peripheral action, and nimodipine, believed to selectively affect cerebral blood flow (Kazda and

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Towart, 1982; Scriabine et al., 1989; De Jong et al., 1990), and a non-dihydropyridine Ca^{2+} channel antagonist, verapamil. The Ca^{2+} channel antagonists were tested both for their ability to prevent expression of the abstinence syndrome in morphine-dependent rats, and to prevent the development of morphine dependence when co-administered with the opiate chronically.

The results suggest that blockade of the voltage dependent L-type Ca^{2+} channels effectively facilitates the analgesic action of morphine without affecting the arterial blood pressure in the rat, that it prevents the behavioral and neurochemical signs of naloxone-precipitated abstinence syndrome, and that morphine does not trigger effectively the processes leading to the development of morphine tolerance and dependence when administered during Ca^{2+} channel blockade.

2. Materials and methods

2.1. Animals

The experiment was carried out on male Wistar rats, weighing 220–250 g, kept under standard laboratory conditions, five to a home cage ($55 \times 35 \times 20$ cm plastic cages with wire lid and sawdust bedding) with free access to standard laboratory food and tap water, at room temperature of approximately 22°C, in a natural day–night cycle.

2.2. Drugs

The following drugs were used: morphine (hydrochloride, Polfa), nifedipine (Polfa), nimodipine (Polfa), verapamil (Sigma), dihydralazine (Nepressolol®, Ciba-Geigy), clonidine (Boehringer Ingelheim), naloxone (Sigma). All drugs were dissolved in physiological saline or (Ca^{2+} channel blockers) suspended in 1% Tween 80, and given in a volume of 4 ml/kg i.p.

2.3. Behavioral tests and procedures

2.3.1. Morphine analgesia

Responsiveness to a painful stimulus was assessed in the hot-plate test (Woolfe and MacDonald, 1944). Briefly, the rats were placed on a metal plate kept at 56°C and surrounded by a translucent cylinder. The latency of response (licking of hind paws or jumping) was recorded. The cut-off time was 30 s.

In acute experiments, morphine was given in doses of 5–12.5 mg/kg, 30 min before the test. Ca^{2+} channel antagonists were given 20 min before morphine injection.

2.3.2. Morphine tolerance

To induce tolerance, morphine was given chronically in a daily dose of 20 mg/kg from day 1 to day 8, alone or following a Ca^{2+} channel antagonist injection, 20 min earlier. In control groups, a Ca^{2+} channel antagonist or saline was given according to the same schedule. The hot-plate test was carried out on days 1, 4 and 8 of the experiment, 30 min after the morphine injection (saline in the controls).

2.3.3. Abstinence syndrome

To investigate dependence, morphine was given chronically in a daily dose of 20 mg/kg for 5 consecutive days, followed by 30 mg/kg for the next 3–5 days, till the evident abstinence signs (i.e., over 10 head twitches during 30 min of observation and hyperreactivity to touch) appeared in the animals 24 h after the last injection.

Rats tested for the abstinence syndrome were weighed, injected with 2 mg/kg naloxone, and placed in a wire-mesh cage. For the following 60 min the occurrence of head or body shakes was recorded by two independent observers, and the rats were then weighed again to record body weight loss, which was caused by intense diarrhea.

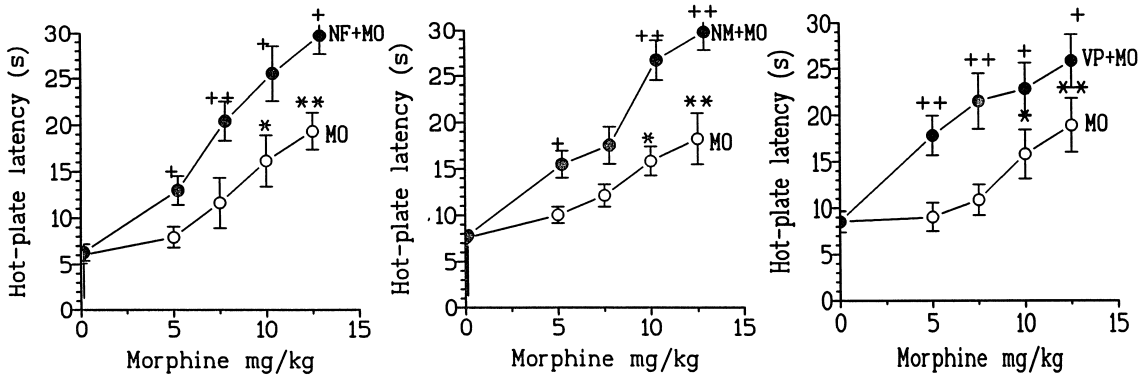

Fig. 1. Potentiation of morphine analgesia by Ca^{2+} channel antagonists in the hot-plate test. MO, morphine; NF, nifedipine 5 mg/kg; NM, nimodipine 5 mg/kg; VP, verapamil 10 mg/kg. The symbols represent means ± S.E.M. from 8 animals. Abscissa, morphine dose (mg/kg); ordinate, hot-plate latency(s). Asterisks indicate a significant analgesia in the morphine group ($* P < 0.05$, $** P < 0.01$). Crosses indicate the significance of difference between the group receiving morphine with and without a Ca^{2+} antagonist ($+P < 0.05$, $++P < 0.01$).

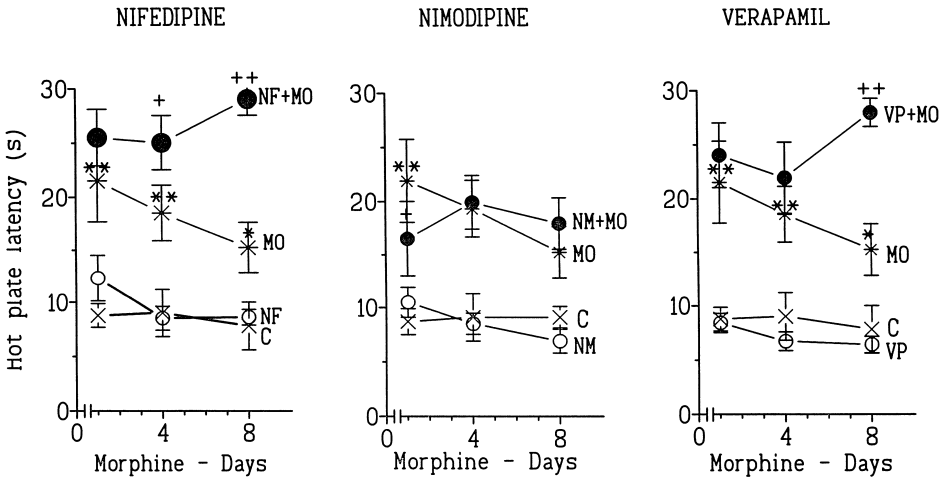

Fig. 2. The effect of Ca^{2+} channel antagonists on the development of tolerance to the analgesic effect of morphine (hot-plate test). Morphine was given in a dose of 20 mg/kg, one daily. C, control group. Abscissa, day of morphine treatment. For other abbreviations and symbols, see Fig. 1.

2.3.3.1. Prevention of expression of abstinence syndrome. The Ca^{2+} channel antagonists were administered once to morphine-dependent rats, approximately 24 h after the last dose of morphine, 20 min before the injection of naloxone.

2.3.3.2. Prevention of development of morphine dependence. The Ca^{2+} channel antagonists were always administered 20 min before each administration of the successive dose of morphine; the last injection of a Ca^{2+} channel antagonist was given, therefore, approximately 24 h before the naloxone injection.

2.4. Blood pressure

Systolic and diastolic blood pressure was measured using a non-invasive blood pressure transducer (Stoelting) connected to a Knauer recorder. The rats that were habituated to the procedure, were hand-held during the measurement and blood pressure was recorded from the tail 1 h after the injection of the Ca^{2+} channel antagonist investigated and the reference hypotensive drugs (dihydralazine, clonidine). Measurements were carried out after the first, seventh, and the last (14th) injection of each Ca^{2+} channel antagonist in the chronic experiment.

2.5. Changes in parameters of [^{3}H]nitrendipine binding sites

2.5.1. Drug treatment

The rats received: nifedipine (5 mg/kg), nimodipine (5 mg/kg) and verapamil (10 mg/kg) once daily for 8 days. Morphine was given for 5 days in a dose of 20 mg/kg per day, and for the next 3 days in a dose of 30 mg/kg. Some of the animals received morphine 20 min after a Ca^{2+} channel antagonist. All rats received naloxone, 2 mg/kg, 24 h after the last injection and were decapitated 90 min later.

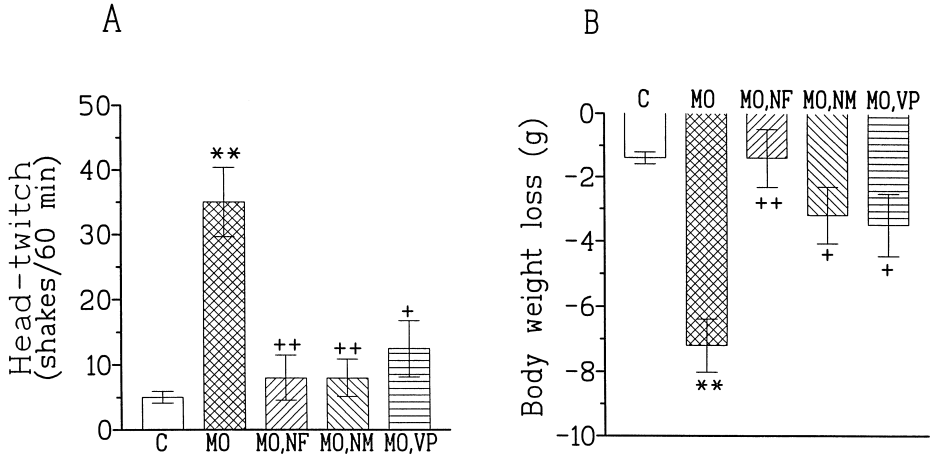

Fig. 3. The influence of Ca^{2+} channel antagonists on expression of naloxone-precipitated morphine abstinence syndrome. (A) Head twitch response (per h), (B) Body weight loss (in g). The rats were made dependent on morphine by 8–10 injections (the controls received saline injections). Twenty-four hours after the last injection, they received placebo (control) or a Ca^{2+} channel antagonist (for abbreviations, see Fig. 1) followed 20 min later by naloxone (2 mg/kg). The tests began immediately after naloxone injection.

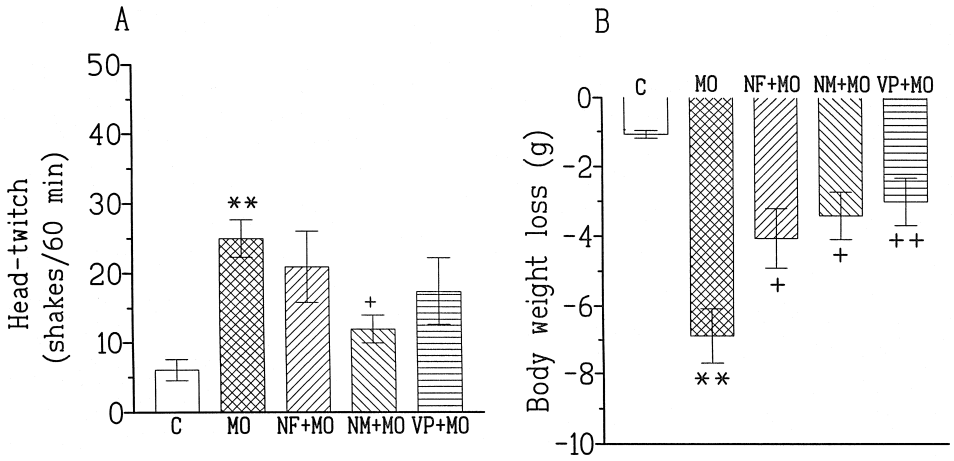

Fig. 4. The influence of Ca^{2+} channel antagonists on development of naloxone-precipitated morphine abstinence syndrome. (A) Head twitch response (per h), (B) Body weight loss (in g). The rats were made dependent on morphine by 8–10 injections (the controls received saline injections); each morphine injection was preceded 20 min earlier by placebo or a Ca^{2+} channel antagonist (for abbreviations, see Fig. 1). Twenty-four hours after the last injection, all rats received naloxone (2 mg/kg).The tests began immediately after naloxone injection.

2.5.2. Membrane preparation and assay of [^{3}H]nitrendipine binding

The experiment was carried out as described previously (Antkiewicz-Michaluk et al., 1990). The brain was rapidly removed after decapitation, placed on an ice-chilled porcelain plate, and the cerebral cortices were dissected out. The tissues were homogenized using a Polytron disintegrator (setting 4, 10 s) at 0°C in 20 volumes 50 mmol/l Tris–HCl buffer, pH 7.6. The cortex from each animal was homogenized separately.

The homogenate was centrifuged at 0°C and $1000 \times g$ for 10 min, the supernatant was decanted and recentrifuged at 0°C and $25\,000 \times g$ for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The pellet thus obtained (fraction P2 of Whittaker and Barker, 1972) was stored at −18°C for no longer than 48 h. For incubation it was reconstituted in the Tris–HCl buffer to give a final protein concentration (measured according to Lowry et al., 1951) of approximately 1.2 mg/ml.

Incubation was carried out in duplicate, in a shaking water bath, at 25°C for 30 min. The radioligand, [^{3}H]nitrendipine (NEN, specific activity 78.3 Ci/mmol), was prepared in the dark in six concentrations (0.05–3 nM) in the buffer. The incubation mixture (final volume 550 μl) consisted of 450 μl membrane suspension, 50 μl of a [^{3}H]nitrendipine solution and 50 μl buffer without (total binding) or with (unspecific binding) nifedipine (final concentration 10 μM). Addition of the radioligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer and placed in plastic scintillation minivials. The scintillation fluid (Aquascint) was added and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter.

In all experiments, specific binding was defined as the difference between total and non-specific binding, and was expressed in fmol/mg protein.

2.6. Statistics

Analysis of variance followed by Dunnett's test (with harmonic means for unequal groups) or the Least Significant Difference test was used to assess the significance of differences between groups.

3. Results

3.1. Morphine analgesia

The analgesic effect of morphine in the hot-plate test was observed after doses of 10 mg/kg and higher. All Ca^{2+} channel antagonists tested, which were used in doses of 5 mg/kg (dihydropyridines) or 10 mg/kg (verapamil) enhanced the analgesic effect of effective doses of morphine and significant analgesia was also produced when morphine doses ineffective given alone, were combined with Ca^{2+} channel antagonists (Fig. 1).

Table 1
Lack of effect of chronic treatment with Ca^{2+} channel antagonists on the binding parameters of cortical [^{3}H]nitrendipine binding sites

Treatment, daily dose (mg/kg)	B_{max} (fmol/mg protein)	%	K_d (nmol/l)	%
Placebo	97 ± 7	100	1.6 ± 0.2	100
Nifedipine, 5	120 ± 8	124	1.7 ± 0.2	106
Nimodipine, 5	92 ± 5	95	1.4 ± 0.1	87
Verapamil, 10	121 ± 8	125	1.8 ± 0.3	112

The rats received the treatment for 8 days and were killed 24 h after the last injection. All groups consisted of 8 animals. The data are means ± S.E.M. No result differed significantly from the control values.

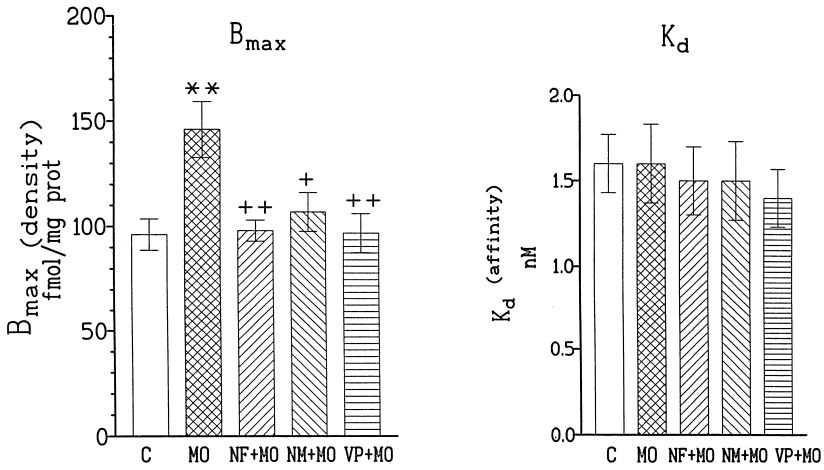

Fig. 5. The inhibition by Ca^{2+} channel antagonists of morphine abstinence-induced up-regulation of [^{3}H]nitrendipine binding sites in the cortex of the rat. The rats were made dependent on morphine by 8 injections (the controls received saline injections); each morphine injection was preceded 20 min earlier by placebo or a Ca^{2+} channel antagonist (for abbreviations, see Fig. 1). Twenty-four hours after the last injection, all rats received naloxone (2 mg/kg) and were decapitated 90 min later. Each bar represents the mean ± S.E.M. of 8 individual assays.

3.2. Morphine tolerance

After chronic administration of a high dose of morphine (20 mg/kg) for 8 days, significant tolerance to its analgesic effect developed. In rats, in which each morphine injection was preceded by nifedipine and verapamil, no tolerance appeared at the end of the experiment. In contrast, nimodipine (5 mg/kg) did not prevent the development of tolerance (Fig. 2).

3.3. Morphine abstinence

3.3.1. Prevention of expression of abstinence syndrome

Naloxone injection to morphine-naive (placebo-treated) rats did not produce more head twitches than observed in controls, and no signs of diarrhea were seen in these rats. Morphine-dependent rats that were given naloxone following placebo injection showed prominent wet-dog shakes and head twitches, as well as diarrhea that resulted in a significant body weight loss. The injections of Ca^{2+} channel antagonists, nifedipine (5 mg/kg), nimodipine (5 mg/kg) and verapamil (10 mg/kg) 20 min before the injection of naloxone to morphine-dependent rats prevented the head twitch response (Fig. 3A) and strongly reduced the body weight loss caused by diarrhea (Fig. 3B).

3.3.2. Prevention of development of morphine dependence

The abstinence syndrome, produced by naloxone, given 24 h after the last morphine injection in rats that received morphine chronically together with nimodipine or verapamil was reduced, as demonstrated by inhibition of the body weight loss (Fig. 4B). The head twitch response, however, was not significantly reduced; only nimodipine had a protective effect with a borderline significance ($0.1 > P > 0.05$) (Fig. 4A).

3.4. [^{3}H]nitrendipine binding sites

Chronic administration of Ca^{2+} channel antagonists did not change the binding parameters of the [^{3}H]nitrendipine

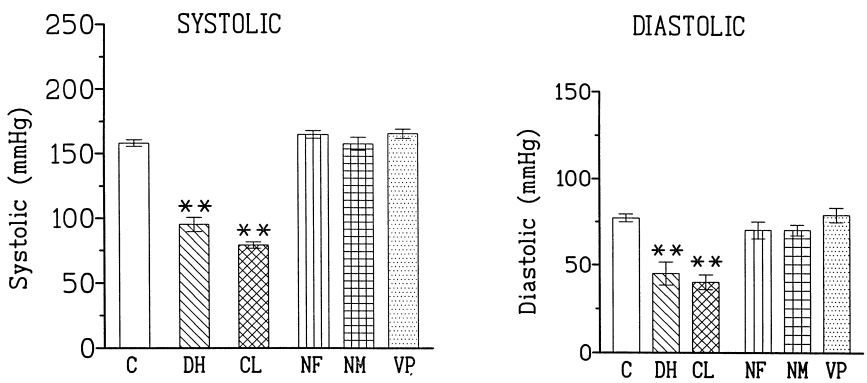

Fig. 6. The effect of Ca^{2+} channel antagonists and reference hypotensive drugs on arterial blood pressure in the rat. The hypotensive drugs were given 1 h after the injection of saline (control), Ca^{2+} channel antagonists (for abbreviations, see Fig. 1), dihydralazine, 4 mg/kg (DH), or clonidine, 2 mg/kg (CL).

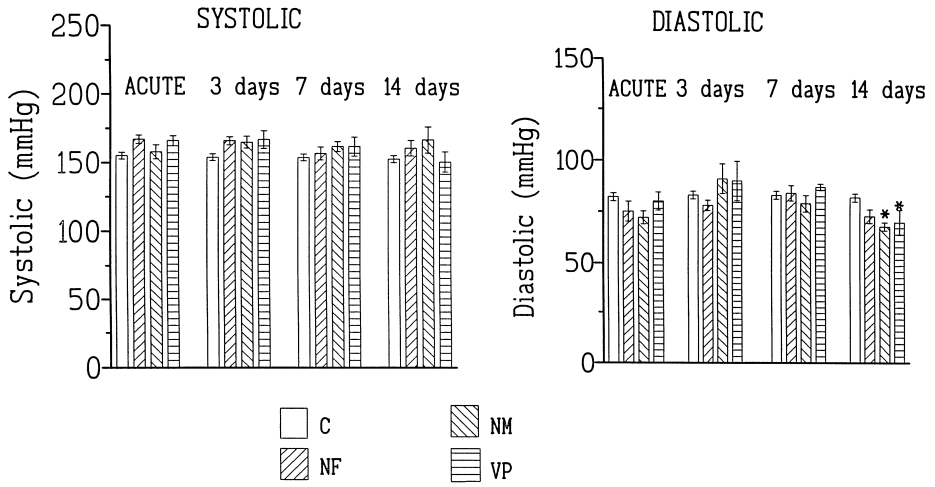

Fig. 7. The effect of chronic administration of Ca^{2+} channel antagonists on arterial blood pressure in the rat. The drugs were given for 14 days, the measurements were made on days 1, 7 and 14, always 1 h after the last injection. For abbreviations, see Fig. 1.

binding sites (Table 1). In the cortex of rats killed during the naloxone-induced morphine abstinence syndrome, the density of [^{3}H]nitrendipine binding sites was significantly elevated, by approximately 30%. In the rats receiving the investigated Ca^{2+} channel antagonists before each morphine injection, the abstinence-induced up-regulation of cortical Ca^{2+} channels was completely prevented (Fig. 5).

3.5. Blood pressure

Where given acutely, classical hypotensive drugs, dihydralazine and clonidine, significantly depressed both the systolic and diastolic arterial pressure in conscious rats, none of the Ca^{2+} channel antagonists tested produced any change in the arterial blood pressure (Fig. 6). Also, chronic administration of the Ca^{2+} channel antagonists, for 3 and 7 days, produced no change in blood pressure: after chronic administration of nimodipine and verapamil for 14 days a slight (approximately by 15%), though significant, decrease in the diastolic pressure was the only change observed (Fig. 7).

4. Discussion

The main finding of this study was that three different Ca^{2+} channel antagonists in a similar manner modify the effects of acutely administered morphine, which suggests that the Ca^{2+} channel blockade, rather than specific properties of the drugs, was the cause of this effect. Single doses of dihydropyridine compounds of low and high lipophilicity (and presumably low and high penetrability to the central nervous system), nifedipine and nimodipine, as well as a phenylethylalkylamine derivative, verapamil, similarly enhance the morphine analgesia and prevent the precipitation by naloxone of the abstinence syndrome in morphine-dependent rats. Some differences among the Ca^{2+} channel antagonists were observed in their action on the development of tolerance and dependence. Given chronically, concomitantly with morphine, nifedipine and verapamil, but not nimodipine, attenuate the development of tolerance to morphine analgesia. All three Ca^{2+} channel antagonists inhibited the development of dependence on morphine, as reflected by prevention of gastrointestinal and biochemical effects of naloxone administration in rats receiving high doses of morphine while they did not prevent the head twitch response.

Several authors have reported that acute administration of Ca^{2+} channel antagonists augments the analgesic effect of morphine (Antkiewicz-Michaluk et al., 1991, 1993; Dierssen et al., 1990; Hoffmeister and Tettenborn, 1986; Kavaliers, 1987; Contreras et al., 1988; Omote et al., 1993), although the effect was dependent on the time of day (Hodoglugil et al., 1996), and was species-specific (Schnur et al., 1992). We have previously reported that nifedipine also exerts its potentiating effect on morphine analgesia in morphine-tolerant rats (Antkiewicz-Michaluk et al., 1993). In the present experiment, we tested the effect of concomitant administration of Ca^{2+} channel antagonists with high doses of morphine to assess the possibility of interference of Ca^{2+} channel blockade with the development of tolerance. Both nifedipine and verapamil were effective in morphine-tolerant rats. However, nimodipine, which in non-tolerant rats was as effective as other Ca^{2+} channel antagonists to potentiate morphine analgesia, failed to prevent the development of tolerance. The latter result is in a perfect agreement with the findings of Dierssen et al. (1990), that nimodipine given concurrently with sufentanil does not prevent the development of tolerance in the tail-flick test. This does not exclude the possibility of using nimodipine in tolerant subjects, as the expression of analgesia was enhanced (Dierssen et al., 1990). In fact, nimodipine has been successfully used for reduction of daily dosage of morphine in cancer patients

(Santillan et al., 1994). It should be noted that Ca^{2+} channel antagonists also enhance the analgesic action of non-opioid compounds, such as prolactin (Ramaswamy et al., 1986), flavonoids (Thirugnanasambantham et al., 1988) and antidepressants (Antkiewicz-Michaluk et al., 1991). Moreover, Ca^{2+} channel antagonists themselves produce an analgesic action (Del Pozo et al., 1987, 1990; Miranda et al., 1992) Therefore, the action of Ca^{2+} channel antagonists may not be directed at the opioid receptor, but be related to the central role of Ca^{2+} in pain transmission (Ben-Sreti et al., 1983; Chapman and Way, 1982; Ross and Cardenos, 1979).

We have previously reported (Antkiewicz-Michaluk et al., 1990) that cortical voltage-dependent Ca^{2+} channels are up-regulated in rats showing signs of morphine withdrawal. The changes are slight 24 h after the last dose of morphine, but are strongly expressed in rats with naloxone-induced abstinence syndrome. This has been confirmed both in the present experiment and by other authors (Littleton and Brennan, 1993; Zharkovsky et al., 1993), and may suggest that such up-regulation may be a neurochemical marker of the abstinence syndrome. In addition, we have now found that all the Ca^{2+} channel antagonists investigated given concomitantly with morphine, completely prevent the naloxone-induced up-regulation of [^{3}H]nitrendipine binding sites. This suggests that morphine administered during L-type Ca^{2+} channel blockade is unable to induce changes related to some neurochemical effects underlying the abstinence syndrome. Moreover, this suggests that all three Ca^{2+} channel antagonists used in this experiment, in spite of different affinities to the central nervous system structure (Rosenberg, 1991), have a central component of action.

The present study extends our previous finding with nifedipine (Antkiewicz-Michaluk et al., 1990, 1993) that co-administration of a Ca^{2+} channel antagonist with morphine in a chronic experiment attenuates the development of morphine dependence, as reflected by attenuation of the naloxone-induced abstinence syndrome. It should be noted that the abstinence-induced diarrhea was more effectively prevented by concomitant administration of Ca^{2+} channel antagonists than were the head twitches. This may suggest that the course of development of dependence of opioid receptors in the gastrointestinal tract differs from that in the central nervous system. In this study, the schedule of morphine treatment used to obtain dependence involved higher doses and lasted longer than in our previous study (Antkiewicz-Michaluk et al., 1993) to insure the full development of dependence. With this schedule, nifedipine, previously shown to block completely the development of morphine dependence, was the least effective of all three Ca^{2+} channel antagonists, as none of its effects reached significance, while nimodipine and verapamil were effective to prevent full development of peripheral aspects of the abstinence syndrome. Despite those differences, nifedipine, similarly to nimodipine and verapamil, prevented the up-regulation of cortical Ca^{2+} channels during the abstinence syndrome. The results thus show that prevention of up-regulation of cortical voltage-dependent Ca^{2+} channels is not a condition sufficient to prevent the behavioral abstinence syndrome. However, functional integrity of the voltage-dependent Ca^{2+} channels is necessary for expression of the behavioral abstinence syndrome: the investigated Ca^{2+} channel antagonists uniformly prevent the expression of the abstinence syndrome when given directly before administration of naloxone. The present results thus indicate a critical role of the voltage-dependent Ca^{2+} channels both in analgesia and in the acute abstinence syndrome, while the development of tolerance and dependence seems to involve Ca^{2+} channels, but to be regulated in a more complex manner.

Our results support the suggestions of other authors that Ca^{2+} channel antagonists inhibit the development of dependence on several addictive substances. Thus, Dolin and Little (1989), Little et al. (1993), Whittington et al. (1991) has demonstrated that Ca^{2+} channel antagonists prevent the development of alcohol tolerance and dependence and Kuzmin et al. (1992a,b) reported the inhibitory effect of Ca^{2+} channel antagonists on the development of morphine and cocaine dependence. The inhibitory effects of Ca^{2+} channel antagonists on development of dependence were described both in in vivo and in vitro models (Littleton and Brennan, 1993).

The point has sometimes been raised that the modification by Ca^{2+} channel antagonists of behavioral responses to psychotropic drugs might be related to their presumptive hypotensive action. The present results suggest that this is unlikely as the blood pressure changes, induced by the Ca^{2+} channel antagonists tested even after a chronic treatment, are negligible. This finding extends the observations of others, who reported only slight, if any, hypotension in normotensive conscious or anesthetized rats receiving a single dose of a Ca^{2+} channel antagonist (Ishii et al., 1980; Sharma et al., 1984). In this respect Ca^{2+} channel antagonists are different from other hypotensive drugs, dihydralazine and clonidine, that produced a prompt and deep fall in the arterial blood pressure in our experiments.

The results confirm the notion that L-type Ca^{2+} channels play an important role in pain transmission and in processes of adaptation to chronic administration of psychotropic drugs. We have previously reported that applying transient Ca^{2+} channel blockade before administration of haloperidol prevents the development of the withdrawal syndrome (Mamczarz et al., 1994; Antkiewicz-Michaluk et al., 1995) and that lysergic acid diethylamide (LSD-25) administered chronically under conditions of Ca^{2+} channel blockade did not produce the characteristic changes in dopamine metabolism observed after the end of the treatment (Antkiewicz-Michaluk et al., 1997).

The present results suggest that investigation of a possible clinical application of Ca^{2+} channel antagonists should be carried out to test their usefulness to augment the acute

analgesic action of morphine, to attenuate the acute morphine abstinence syndrome, and to be used as adjuvants for the opioid treatment of chronic pain conditions both to prevent the necessity of augmenting the dose and to reduce the risk of development of morphine dependence.

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