Full Paper

Role of Pharmacokinetic Effects in the Potentiation of Morphine Analgesia by L-Type Calcium Channel Blockers in Mice

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Abstract. The present study was designed to investigate the pharmacokinetic interaction of morphine with three classes of L-type calcium channel blockers (CCB) and its relationship to morphine-induced mechanical antinociception in mice. The CCB classes were benzothiazepine (diltiazem), dihydropyridine (nimodipine), and phenylalkylamine (verapamil). Each of the three classes of L-type CCB (diltiazem, 40 and 80 mg/kg; nimodipine, 40 mg/kg; verapamil, 40 mg/kg), when administered prior to morphine (4 mg/kg, s.c.), potentiated the analgesic effect of morphine and markedly increased the level of morphine in serum. Pretreatment with diltiazem (40 and 80 mg/kg) and verapamil (40 mg/kg) also increased morphine level in the brain. However, these drugs produced less increase in morphine level in the brain than they produced in serum (i.e., they decreased the brain-to-serum ratio of morphine). Pretreatment with nimodipine (40 mg/kg) did not affect the morphine level in the brain and also decreased the brain-to-serum ratio of morphine. When morphine (3.2–100 mg/kg, s.c.) was injected alone, the brain-to-serum ratio of morphine was constant, regardless of the morphine dose. These results suggest that increases in morphine concentration in peripheral blood may be, at least in part, involved in the ability of L-type CCBs to potentiate the analgesic effect of morphine.

Keywords: morphine analgesia, diltiazem, nimodipine, verapamil, pharmacokinetics

Introduction

L-type calcium channel blockers (CCB) of the dihydropyridine (nimodipine), benzothiazepine (diltiazem), and phenylalkylamine (verapamil) classes have been reported to modulate opioid effects, including analgesia, tolerance, and dependence (1–3). Several reports have demonstrated that L-type CCBs potentiated the analgesic effects of morphine (4–7). The mechanism of this potentiation has been proposed to be through an L-type CCB-induced reduction in intracellular Ca^{2+} levels, resulting in enhanced release of nociceptive neurotransmitters in the central nervous system.

It is possible, however, that the interaction between

morphine and L-type CCBs is a pharmacokinetic rather than a pharmacodynamic one. L-type CCBs have been reported to be substrates for the cytochrome P450 (CYP) 3A subfamily (8–10), and some of the administered morphine is metabolized to the *N*-demethylated form in rodents, possibly by the CYP 3A subfamily (11, 12). Thus, these two classes of compound could be substrate competitors for CYP 3A. On the other hand, some L-type CCBs and morphine are both substrates for P-glycoprotein (P-gp), which acts as a drug efflux pump at the blood brain barrier (13, 14). Recent reports have demonstrated that morphine levels in brain were increased when it was administered along with other substrates for P-gp (15). These findings raise the possibility that modification of morphine metabolism and/or distribution by L-type CCBs may be involved in their potentiating effect on morphine analgesia.

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In the present study, we examined the effects of three classes of L-type CCBs, nimodipine, diltiazem, and verapamil, on morphine analgesia and pharmacokinetics.

Materials and Methods

Animals

Male ICR mice (25–35 g) were obtained from SLC (Osaka). The animals were housed at a temperature of 23–24°C with a 12 h light-dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were available ad libitum.

The present study was conducted in accordance with the Declaration of Helsinki and with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by The Japanese Pharmacological Society.

Morphine analgesia

The analgesic effect of morphine was measured by the tail-pinch method (16), which measures mechanical antinociception. A flattened clip (approximately 6-mm-wide) was placed at the base of the tail. The pressure produced by the clip on the tail was adjusted to 500 g. A nociceptive response was indicated by the time (latency) required for the mouse to respond to this pressure by vocalizing or biting at the clip. The clip was never applied for longer than 15 s. The percent maximal possible effect (%MPE) was calculated using the following formula: $\%MPE = 100 \times (\text{each latency} - \text{baseline latency}) / (15 - \text{baseline latency})$.

The clip was applied prior to and every 15 min following administration of 4 mg/kg morphine s.c. over a 120-min observation period. This covered the period of morphine analgesia both with and without administration of the L-type CCBs. Several doses of each of the L-type CCBs were administered 30 min before morphine administration to determine changes in the peak analgesic effect of morphine. A time-action curve was constructed and the area under the curve (AUC) was used as the primary indicator of the behavioral interaction between morphine and the L-type CCBs. In addition, AUC measures of the effect of diltiazem (40 mg/kg) on analgesia produced by a range of doses of morphine (0.62, 1.25, 2.5 and 5.0 mg/kg) were taken.

Morphine content

Separate groups of mice were used to determine the pharmacokinetic interaction between morphine and the L-type CCBs. The same doses and timing of drug administration were used as had been used in the analgesia study. Blood and brain samples were collected 45 min after morphine administration. Serum was separated by centrifugation (2000 × g for 20 min at 4°C).

The brain was homogenized in 1 ml of pure water by sonication (20 s). The mixture of sample (100 µl of serum or brain homogenate) and 40% K₂HPO₄ solution (1 ml) was shaken with 5 ml of ethylacetate for 20 min and then centrifuged at 2000 × g for 5 min at 4°C. The organic layer was collected, and the aqueous layer was re-extracted with 5 ml of ethylacetate. Then, the morphine in the organic layer was extracted with 1 ml of 1 M acetic acid, and a 0.9 ml portion of the aqueous layer was lyophilized. The samples were dissolved in 200 µl of 0.01 M HCl, and 20 µl was analyzed by the HPLC-ECD system. The condition of the HPLC-ECD system were as follows: column, Eicompak MA-ODS (Eicom, Kyoto); mobile phase, 0.1 M citric acetate buffer (pH 3.9)/methanol (82/18) containing 3 mg/l of EDTA and 150 mg/l of sodium octane sulfonate; flow rate, 1 ml/min; detector, ECD-100 (Eicom) 750 mV Ag/AgCl; temperature, 25°C.

Drugs

The drugs used and their suppliers were as follows: morphine hydrochloride (Takeda Chemical Industries, Ltd., Osaka); diltiazem, verapamil, and nimodipine (Sigma, St. Louis, MO, USA). Morphine, diltiazem, and verapamil were dissolved in saline and administered subcutaneously. Nimodipine was suspended in 40% polyethylene glycol 400/saline and administered intraperitoneally.

Statistical analyses

Data are presented as the mean with S.E.M. The statistical significance was assessed with a one way analysis of variance followed by the Student-Newmann-Keuls test. The differences were regarded as significant when *P* values were less than 0.05. To evaluate the potentiating effect of diltiazem on morphine analgesia, parallel dose-response lines were constructed using a computer program (17).

Results

Morphine at 4 mg/kg, s.c. produced an analgesic response in the mice that reach a peak of approximately 75%MPE 30 min following morphine administration and lasted for about 105 min (Fig. 1). The administration of 80 mg/kg of diltiazem by itself (diltiazem, 80 mg/kg + SAL) did not produce an antinociceptive effect. However, the administration of diltiazem at 40 or 80 mg/kg prior to morphine produced a dose-related increase in the peak response of morphine to nearly 100%MPE. The analgesic effect of morphine also had a faster onset when it was preceded by these doses of diltiazem, but it did not last longer (Fig. 1A). As deter-

Fig. 1. Effects of diltiazem on analgesic effect of morphine. A: Time-course of analgesia. B: Area under the analgesic curve (AUC). Mice received diltiazem (DIL; 20, 40, and 80 mg/kg, s.c.) or saline (SAL) 30 min before morphine (MOR; 4 mg/kg, s.c.) administration. Each column represents the mean with S.E.M. of 5 mice. *Vs* SAL + morphine, 4 mg/kg, **P < 0.01. %MPE: percent maximal possible effect.

mined by AUC, pretreatment with 40 and 80 mg/kg of diltiazem significantly enhanced the analgesic effect of morphine (Fig. 1B). Evaluation of the effect of 40 mg/kg diltiazem on several doses of morphine indicated that pretreatment with diltiazem shifted the morphine dose-response curve about 1.8-fold to the left (Fig. 2).

The administration of 40 mg/kg of nimodipine (Fig. 3A) or verapamil (Fig. 3B) alone did not produce an antinociceptive effect. However, pretreatment with 40 mg/kg of nimodipine (Fig. 3A) and verapamil (Fig. 3B) significantly potentiated the analgesic response to 4 mg/kg morphine.

The administration of diltiazem (40 and 80 mg/kg), nimodipine (40 mg/kg), and verapamil (20 and 40 mg/kg) prior to morphine significantly increased the amount of morphine in serum (Fig. 4A). Pretreatment with diltiazem (40 and 80 mg/kg) and verapamil (40 mg/kg) also significantly increased morphine level in the brain, although nimodipine had no effect in brain levels of morphine (Fig. 4A). However, pretreatment

Fig. 2. The potency of diltiazem-induced potentiation of morphine analgesia. Open circle: 1.25, 2.5, and 5 mg/kg of morphine (MOR) in combination with saline (SAL). Closed circle: 0.625, 1.25, and 2.5 mg/kg of morphine in combination with 40 mg/kg of diltiazem (DIL). Mice received diltiazem (s.c.) or saline (s.c.) 30 min before morphine (s.c.) administration. Each circle represents the mean with S.E.M. of 5 mice.

Fig. 3. Effects of nimodipine and verapamil on analgesic effect of morphine. A: Mice received nimodipine (20 and 40 mg/kg, i.p.) or 40% polyethyleneglycol (PEG) 30 min before morphine (4 mg/kg, s.c.) administration. Each column represents the mean with S.E.M. *Vs* PEG + morphine 4 mg/kg, **P < 0.01. B: Mice received verapamil (20 and 40 mg/kg, s.c.) or saline (SAL) 30 min before morphine (4 mg/kg, s.c.) administration. Each column represents the mean with S.E.M. *Vs* SAL + morphine 4 mg/kg, **P < 0.01. The number in parentheses indicate the number of mice.

Fig. 4. Effects of L-type CCBs on morphine level in brain and serum. A: Morphine level in brain and serum. B: The brain-to-serum ratio of morphine level. Mice received diltiazem (DIL; 40 and 80 mg/kg, s.c.), nimodipine (NIM; 20 and 40 mg/kg, i.p.), and verapamil (VER; 20 and 40 mg/kg, s.c.) and their respective vehicles (SAL or PEG) 30 min before morphine (4 mg/kg, s.c.) administration. Blood and brain were collected 45 min after morphine administration. Each column represents the mean with S.E.M. The number in parentheses indicate the number of mice. *Vs* respective vehicles (SAL or PEG), * $P < 0.05$, ** $P < 0.01$.

with all three L-type CCBs produced dose-dependent decreases in the brain-to-serum ratio of morphine (Fig. 4B), indicating that L-type CCBs-induced much higher increases in morphine levels in serum than in brain. Administration of morphine alone (3.2–100 mg/kg) increased morphine levels in both brain and serum, proportionally to the doses of morphine (Fig. 5A), showing that the brain-to-serum ratio of morphine level was constant regardless of the dose of morphine (Fig. 5B).

Discussion

Consistent with previous reports, the present study shows that diltiazem, nimodipine, and verapamil enhanced the analgesic effect of morphine. Interestingly,

Fig. 5. Effects of several doses of morphine on morphine level in brain and serum. A: Morphine level in brain and serum. B: The brain-to-serum ratio of morphine level. Blood and brain were collected 45 min after morphine (3.2–100 mg/kg, s.c.) administration. Each column represents the mean with S.E.M. The number in parentheses indicate the number of mice.

the potentiating effect of these three L-type CCBs on morphine analgesia was accompanied by marked increases in morphine level in the serum but not in the brain.

The major two pathways for the metabolism of morphine in rodents are glucuronidation to morphine-3-glucuronide (M-3-G) and *N*-demethylation to normorphine with subsequent conjugation to normorphine-3-glucuronide (18, 19). Morphine glucuronidation is catalyzed by uridine diphosphate-glucuronosyltransferase, and morphine *N*-demethylation is catalyzed possibly by the cytochrome CYP3A subfamily (11, 12). The L-type CCBs used in the present study have been reported to be substrates for the CYP3A subfamily (8–10) and thus may act as competitive inhibitors of this metabolizing enzyme.

On the other hand, L-type CCBs have been used as hypotensive drugs. Previous reports demonstrated that systemic administration of smaller doses of L-type

CCBs than those used in the present study could produce hypotensive effects (20–22). This raises the possibility that L-type CCBs-induced hypotension leads to decrease in the hepatic blood flow. In our pilot study, the hypotensive drug reserpine (5 mg/kg, i.p., 24 h before morphine injection) also had a similar effect to L-type CCBs (data not shown); this may support the possibility. Therefore, the increase in morphine levels in serum that was observed in the present study may be attributed either to disturbance of the CYP3A-mediated metabolic pathway for morphine and/or decreases in metabolic rate due to decreases in hepatic blood flow.

It is expected that increases in morphine concentration in serum will lead to increases in its content in the brain. In fact, when morphine was injected alone (3.2–100 mg/kg), the brain-to-serum ratio of morphine level was constant regardless of the morphine dose. In our results, however, L-type CCBs-induced increases in morphine level in brain were much lower than those in serum (i.e., decreased brain/serum ratio of morphine). It appears that penetration of morphine into the brain was suppressed by the L-type CCBs.

Recently, P-gp, which acts as a drug efflux pump, was identified as an important determinant of drug permeability across the blood-brain barrier (23). All three L-type CCBs used in the present study have been reported to be substrates of P-gp, although the potencies of three L-type CCBs as inhibitors of P-gp are different (14). Morphine is also a substrate of P-gp, and recent *in vivo* studies using P-gp deficient mice demonstrated that morphine levels in the brain after systemic administration of morphine were higher in P-gp deficient mice than in wild-type mice (24, 25). These results indicate that P-gp serves to reduce the penetration of morphine into the brain. Therefore, L-type CCBs might be expected to increase morphine penetration into the brain by competing with P-gp. In fact, Zong and Pollack (24) demonstrated that the brain-to-serum ratio of morphine was increased by treatment with verapamil, in contrast to the results shown here. On the other hand, Cisternino et al. (25) reported that pretreatment with P-gp inhibitors GF120918, PSC833, or verapamil failed to increase the brain uptake of morphine in mice. Although the reason why such contradictory results were obtained from different investigators remains unclear, differences of experimental conditions, especially the dose of verapamil (20 and 40 mg/kg were used here; Cisternino et al. used 1 mg/kg; Zong and Pollack used 100 mg/kg) may affect these experimental results. There is also the possibility that the L-type CCBs induced dose-related vasodilation that might affect the brain blood flow and perhaps prevent the observation of L-type CCBs-induced inhibition of P-gp.

This study demonstrated that L-type CCBs potentiate morphine analgesia and produce marked increases in morphine levels in serum with considerably less increases in morphine levels in brain. In particular, nimodipine produced increases in morphine levels in serum but had no effect on these levels in brain; nimodipine was nearly as able as the other two L-type CCBs to enhance the analgesic response to morphine. The results of the present study showed that the potency of L-type CCBs-induced increases in serum morphine levels and their potentiation of morphine analgesia were in the same order, that is, the rank order of % increases in serum morphine level over the respective controls were verapamil (164.0%) $\geq$ diltiazem (142.2%) $>$ nimodipine (73.2%), and that of % increases in AUC over the respective controls were verapamil (122.9%) $\geq$ diltiazem (94.4%) $>$ nimodipine (41.0%). These results raise the possibility that peripheral analgesic mechanisms may be involved in the L-type CCBs-induced potentiation of morphine analgesia. Indeed, opioid receptor mRNAs have been found on dorsal root ganglia (26) and opioid receptors also exist on peripheral cutaneous unmyelinated and myelinated axons (27, 28) and produced potent peripheral analgesia (29–36). Further studies are currently underway in our laboratory to elucidate such a possibility.

In summary, we demonstrated that three classes of L-type CCBs, diltiazem, nimodipine, and verapamil, potentiated the analgesic effect of morphine and produced a marked increase in morphine levels in the serum but not in the brain. The present results indicate that a modification of morphine metabolism and distribution may be, at least in part, involved in the ability of L-type CCBs to potentiate morphine analgesia.

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