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European Journal of Pharmacology 397 (2000) 85–92

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Diltiazem enhances the analgesic but not the respiratory depressant effects of morphine in rhesus monkeys

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Received 23 February 2000; received in revised form 16 March 2000; accepted 21 March 2000

Abstract

There is evidence that blockade of Ca^{2+} channels can modify the analgesia and respiratory depression produced by opioid drugs. The interaction between Ca^{2+} channel blockade and drug-induced analgesia and respiratory depression was examined by administration of the L-type Ca^{2+} channel blocker diltiazem together with various analgesic drugs. The antinociceptive effects of the drugs were evaluated using a warm-water (50°C) tail-withdrawal assay in rhesus monkeys, and the respiratory depressant effects were evaluated using a pressure-displacement plethysmograph. Pretreatment with diltiazem (10–40 mg/kg, i.m.) 30 min before administration of morphine (0.3 to 10 mg/kg) or heroin (0.03 to 1.0 mg/kg) produced a dose-dependent potentiation of the opioid-induced analgesia. The analgesic potency of morphine and heroin was increased by approximately 0.5 log unit in the presence of 40 mg/kg diltiazem. However, diltiazem failed to alter the analgesic potencies of the μ-opioid receptor agonists, fentanyl, etonitazene, nalbuphine, the κ-opioid receptor agonist, U-50,488 [(*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide], or the non-opioid, clonidine. Respiratory frequency, minute volume, and tidal volume were suppressed by morphine, heroin, and fentanyl, but these effects were not modified by pretreatment with diltiazem (40 mg/kg). These results suggest that diltiazem selectively potentiates morphine- and heroin-induced analgesia without modifying the effects of these opioids on respiration.

Keywords: Respiration; Ca^{2+} channel blocker; Opioid; Rhesus monkey

1. Introduction

μ-Opioid receptor agonists have a number of important clinical properties. Among others, they are very effective analgesics, and they suppress respiration. There is evidence, some conflicting, that drug-induced blockade of Ca^{2+} channels can modify both of these actions. An intracerebroventricular injection of Ca^{2+} reduced opioid-induced analgesia in mice, whereas a Ca^{2+} chelator (EDTA) potentiated opioid-induced analgesia (Kakunaga et al., 1966). In addition, morphine-induced analgesia was enhanced by administration of the L-type Ca^{2+} channel blockers diltiazem, flunarizine, nicardipine, and verapamil in mice using the hot-plate test (Contreras et al., 1988). Other studies have suggested an influence of Ca^{2+} channel blockade on the development of tolerance to sufentanil's analgesic effects (Diaz et al., 1995a; 1995b). These data indicate that μ-opioid receptor-induced analgesia can be affected by Ca^{2+}, but there are no data on a possibly similar interaction between Ca^{2+} channel blockade and analgesia induced by other drugs.

Carta et al. (1990) reported that the Ca^{2+} channel blocker nifedipine did not affect morphine-induced respiratory suppression, as estimated by blood gas analysis, in humans. On the other hand, nimodipine enhanced respiratory suppression induced by the μ-opioid receptor agonist sufentanil using pneumotachograph measures in anesthetized cats (Dierssen et al., 1991) and whole-body plethysmograph measures in rats (Ruiz et al., 1993). Conversely, verapamil inhibited morphine-induced respiratory suppression in rats (Szikszay et al., 1986). It therefore remains unclear how L-type Ca^{2+} channel blockers interact with opioids in measures of respiration.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00248-X

In this experiment, we examined the effects of diltiazem on analgesia induced in rhesus monkeys by both μ-opioid receptor and κ-opioid receptor agonists as well as by clonidine. The effects of diltiazem on μ-opioid receptor-induced respiratory suppression were evaluated as well.

2. Materials and methods

2.1. Subjects

Fourteen adult rhesus monkeys (*Macaca mulatta*) of either sex, weighing between 4.5 and 15.0 kg, were used for antinociceptive and respiratory suppression assays. They were housed individually in cages in a room with controlled temperature and light cycles (6:00 a.m. to 6:00 p.m. light). Water was available ad libitum, and the monkeys were fed approximately 30 biscuits (Purina Monkey Chow) daily, supplemented twice weekly with fresh fruit. The procedures used in this study were approved by the University of Michigan Committee on the Use and Care of Laboratory Animals.

2.2. Warm-water tail-withdrawal assay

2.2.1. Apparatus and procedure

The procedure used in the present study to evaluate analgesia was similar to that described by Dykstra and Woods (1986). Monkeys were seated in primate restraint chairs and the lower part of their shaved tails (approximately 15 cm) was immersed in a thermos flask containing water maintained at either 40°C, 50°C, or 55°C. Tail-withdrawal latencies were timed manually using a microprocessor (IBM PC) via a hand-held push-button switch. If the monkeys did not remove their tails from the flask within 20 s (cut-off), the flask was removed and a maximum time of 20 s was recorded. Sessions began with control determinations at each of the three water temperatures. The 40°C temperature was used as a non-noxious control. For a subject to be used in subsequent evaluations on that day, it had to leave its tail in the 40°C water for 20 s. The higher temperatures are differentially sensitive to high and low efficacy μ-opioid receptor agonists. Low efficacy agonists produce an increased latency of tail-withdrawal from 50°C but not 55°C water, whereas high efficacy agonists produce an increased tail-withdrawal latency from both of these temperatures (e.g., Walker et al., 1993). Only data from the 50°C water are presented here. In a study of diltiazem effects on the time course of morphine analgesia, diltiazem or vehicle was given 30 min prior to a bolus injection of morphine. Tail-withdrawal latencies were determined at 30- to 60-min intervals after morphine administration. Control tail-withdrawal latencies were obtained before administration of diltiazem, and just prior to administration of morphine.

Dose–response curves for the several analgesics were obtained by a cumulative administration procedure. Thirty-minute cycles were used; 23 min after the start of each cycle, the effects of each of the three water temperatures were tested. The temperatures were presented in varying order with approximately 2 min between each exposure. When the effect of an analgesic was evaluated, no injection was given at the end of the first cycle, and baseline tail-withdrawal measures were taken during the last 7 min of this cycle. The initial administration of the analgesic was given at the beginning of the second cycle. Injections were spaced 30 min apart and doses were increased by one half log unit with each injection. If a pretreatment (diltiazem or vehicle) was given, it was preceded by a no-injection control cycle, and was administered at the start of the second 30-min cycle. The effects of the pretreatment alone were evaluated in the last 23-min of the second cycle, and cumulative administration of the analgesic began at the end of the third cycle and continued for as many as five additional cycles.

2.2.2. Data analysis

Individual monkey's data were converted to percent maximum possible effect (%MPE) by the following calculation: $\%MPE = [(\text{test latency} - \text{control latency})/(20\text{ s cut-off latency} - \text{control latency})] \times 100$. ED_{50} values for each opioid were calculated by linear regression from the mean data, and dose ratios were calculated from ED_{50} values with or without diltiazem treatment. Comparisons were made for the same monkeys across conditions. The ED_{50} values were considered to differ significantly from each other if there was no overlap in their 95% confidence limits.

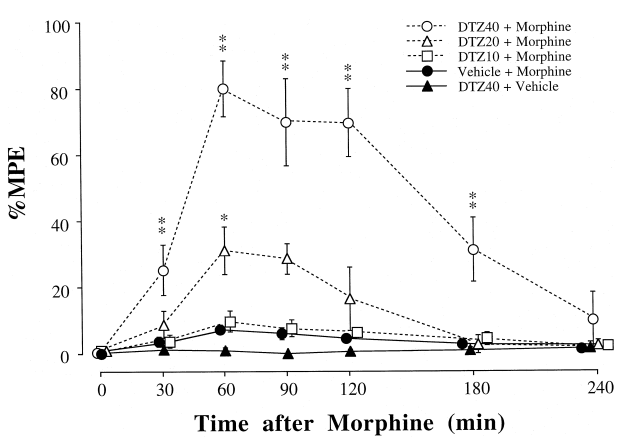

Fig. 1. Time course of morphine-induced analgesia in the presence or absence of diltiazem. The antinociception was estimated using 50°C warm-water tail-withdrawal assay. Diltiazem (10–40 mg/kg, s.c.) was administered 30 min before the morphine (1 mg/kg, s.c.) injection. Each point represents the mean and each vertical bar represents the S.E.M. ($n = 4$). $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Vehicle + Morphine.

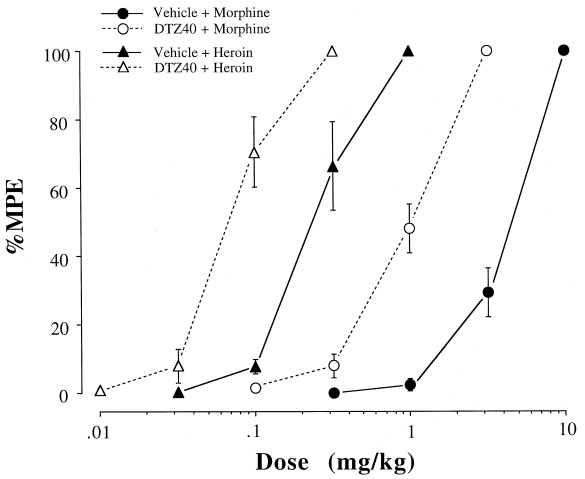

Fig. 2. Dose–response curve of morphine and heroin-induced analgesia in the presence and absence of diltiazem. Morphine and heroin were administered cumulatively. Each point represents the mean and each vertical bar represents the S.E.M. ($n = 4$).

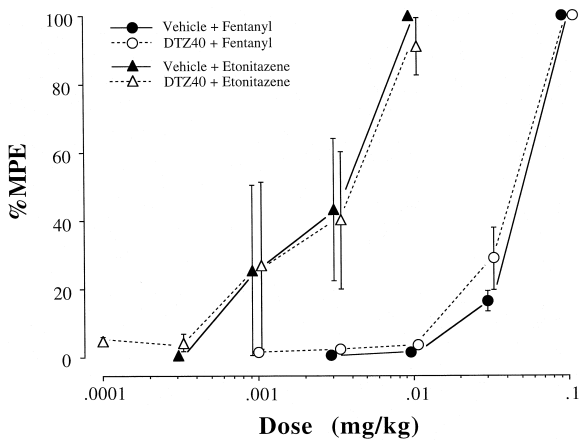

Fig. 3. Dose–response curve of fentanyl- and etonitazene-induced analgesia in the presence and absence of diltiazem (40 mg/kg, s.c.). Fentanyl and etonitazene were administered cumulatively. Each point represents the mean and each vertical bar represents the S.E.M. ($n = 4$).

2.3. Respiration

2.3.1. Apparatus

The apparatus used was similar to that described previously (Howell et al., 1988; France and Woods, 1990; Butelman et al., 1993). Briefly, unanesthetized monkeys were seated in restraint chairs in ventilated, sound-attenuating chambers. Gas [either air or a mixture of 5% CO_2 in air (hereafter referred to as 5% CO_2)] was pumped into the helmet at a rate of 8 l/min and removed at the same rate. The helmet was sealed around the neck of the monkey by two closely fitting latex shields. Changes in pressure inside the helmet produced by ventilation were measured with a pressure transducer connected to a polygraph (Grass Model 7); the data were recorded on a polygraph trace and in a microprocessor via an analog-to-digital converter. The apparatus was calibrated routinely with known quantities of air. Data for consecutive 3-min periods for minute volume (Ve) and respiratory rate (f) were obtained. Tidal volume (Vt) was computed as the ratio Ve/f.

2.3.2. Procedure

Experimental sessions consisted of several consecutive 30-min cycles, each cycle comprising a 23-min exposure to air followed by a 7-min exposure to 5% CO_2. Respiratory measures were taken continuously throughout a session; data from the last 3-min of each 23-min exposure to air and from the second 3-min exposure to 5% CO_2 were used in the analysis. To study the effects of diltiazem on the respiratory depressant effects of various drugs, either

Table 1
ED_{50} values and dose ratios for different opioid and non-opioid analgesics in 50°C water in the presence or absence of diltiazem (40 mg/kg)

Agonists	Conditions	ED_{50} (mg/kg)[a]	(95% C.L.)[b]	Dose ratios[c]
Morphine	Vehicle	4.35	(3.35–5.64)	4.35
	Diltiazem	1.00	(0.64–1.57)	
Heroin	Vehicle	0.250	(0.137–0.454)	3.51
	Diltiazem	0.071	(0.045–0.111)	
Fentanyl	Vehicle	0.050	(0.047–0.054)	1.17
	Diltiazem	0.043	(0.030–0.061)	
Etonitazene	Vehicle	0.0023	(0.0005–0.0108)	0.92
	Diltiazem	0.0025	(0.0005–0.0128)	
Nalbuphine	Vehicle	2.61	(0.15–45.9)	2.00
	Diltiazem	1.31	(0.26–6.45)	
U-50,488	Vehicle	0.66	(0.34–1.27)	1.27
	Diltiazem	0.52	(0.40–0.68)	
Clonidine	Vehicle	0.046	(0.037–0.057)	0.88
	Diltiazem	0.052	(0.047–0.057)	

[a] ED_{50} values are the mean of individual ED_{50}s, which are calculated by least_squares regression from dose–effect curves.
[b] C.L. = confidence limit.
[c] Dose ratios are calculated using corresponding ED_{50} values.

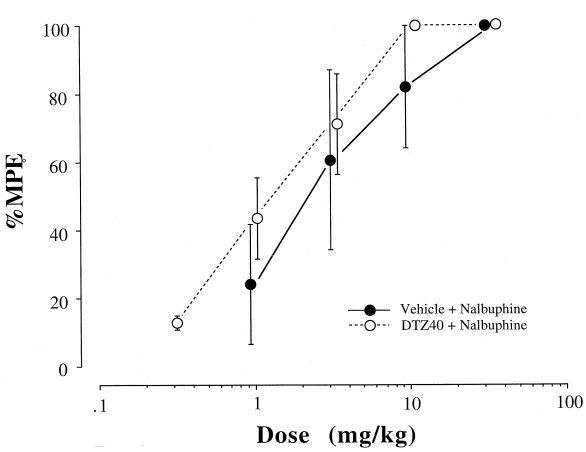

Fig. 4. Dose–response curve of nalbuphine-induced analgesia in the presence or absence of diltiazem (40 mg/kg, s.c.). Nalbuphine was administered cumulatively. Each point represents the mean and each vertical bar represents the S.E.M. ($n = 4$).

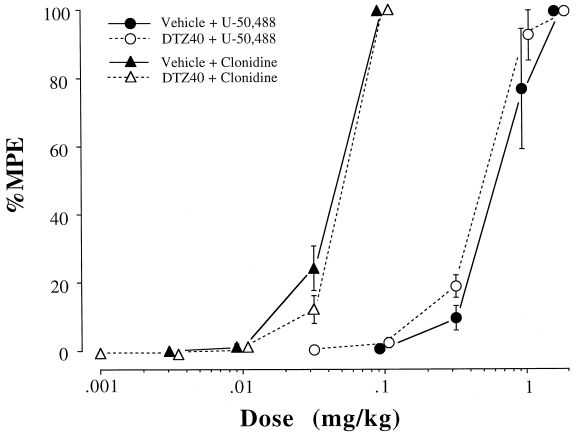

Fig. 5. Dose–response curve of U-50,488 [(*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide]- and clonidine-induced analgesia in the presence or absence of diltiazem (40 mg/kg, s.c.). U-50,488 and clonidine were administered cumulatively. Each point represents the mean and each vertical bar represents the S.E.M. ($n = 4$).

diltiazem or vehicle was administered during the first 3 min of the second cycle (the first cycle was for control observations), and the test drugs were given in cumulatively increasing doses during the first 3 min of subsequent cycles. To study the effect of diltiazem on the time course of morphine-induced respiratory suppression, diltiazem or vehicle was injected during the first 3 min of the second cycle (the first cycle was used for control observations), followed 30 min later by a bolus injection of morphine. Respiratory parameters were measured over the next four cycles.

2.3.3. Data analysis

Values obtained in each experimental session were expressed as percent of control of the respective parameters collected before drug administration. Mean and S.E.M. values were then computed. In the respiration experiments, an ED_{33} (a 33% decrease from control measures) was calculated using procedures described by Butelman et al. (1993). Comparisons were made for the same monkeys across conditions. Data were analyzed by a one-way analysis of variance followed by the Newman–Keuls test for multiple comparisons and Student's *t*-test for comparison between two groups. Analysis of data was done using computer programs described by Tallarida and Murray (1987).

2.4. Drugs

Morphine sulphate, nalbuphine hydrochloride (Mallinckrodt, St. Louis, MO), heroin hydrochloride, fentanyl hydrochloride, etonitazene hydrochloride (National Institute on Drug Abuse, Research Technology Branch, Rockville, MD), U-50,488 [(*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide] (Pharmacia Upjohn, Kalamazoo, MI), clonidine hydrochloride (Boehringer Ingelheim, Ridgefield, CT), and diltiazem

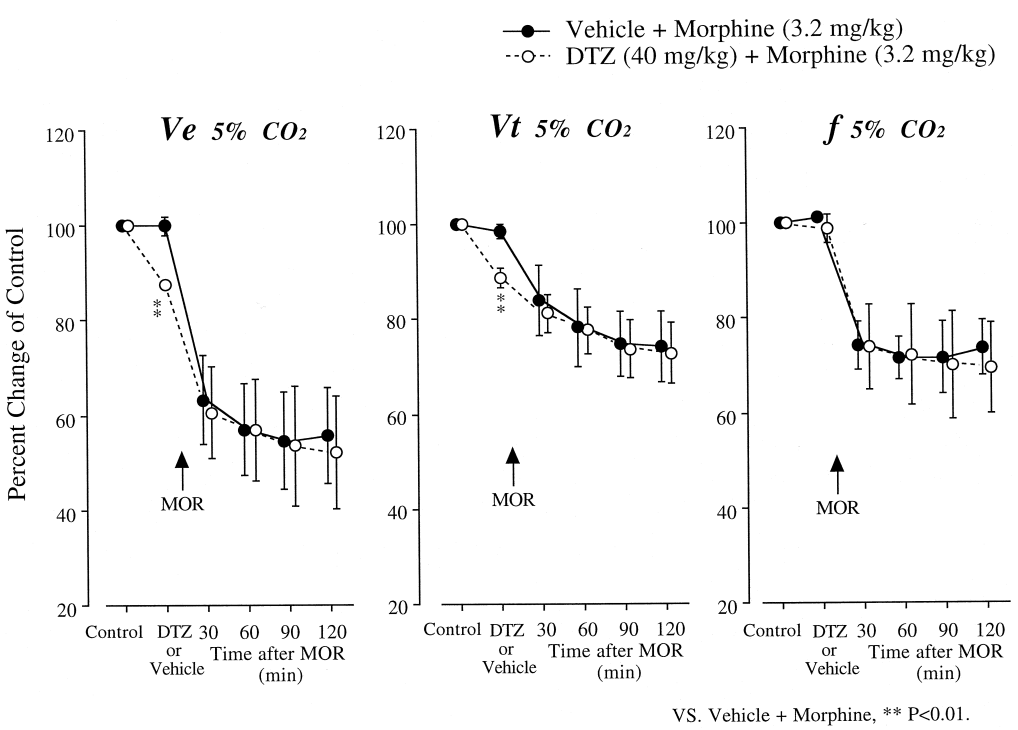

Fig. 6. Time course of a bolus injection of morphine on respiratory parameters in the presence or absence of diltiazem (40 mg/kg, s.c., given 30 min before morphine). Ve = minute volume, Vt = tidal volume, *f* = respiratory frequency in monkeys breathing 5% CO_2 in air. Each point represents the mean and the vertical bars represent the S.E.M. ($n = 4$).

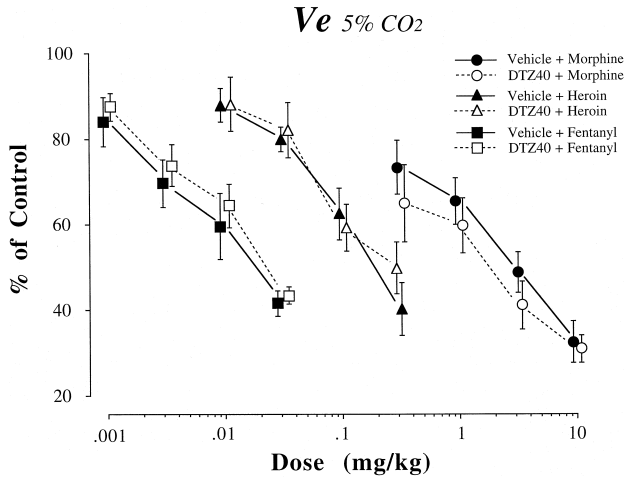

Fig. 7. Dose–response curves of morphine, heroin, and fentanyl estimated using minute volume (Ve). Each of the opioid agonists was administered cumulatively. Each point represents the mean and the vertical bars represent the S.E.M. ($n = 3$ for morphine; $n = 5$ for heroin).

(Sigma, St. Louis, MO) were dissolved in sterile water. All drugs were injected s.c. in the back in the tail-withdrawal assay, and i.m. in the side of the monkeys' thighs in the respiration assay.

3. Results

3.1. Warm-water tail-withdrawal assay

In the absence of drug administration, monkeys displayed a consistent profile of responses in this assay: they left their tails in 40°C water for the 20-s cut-off period, and removed their tails from 50°C water quickly, typically within 2 s. Diltiazem 40 mg/kg alone did not affect tail-withdrawal latencies. Morphine 1 mg/kg produced a slight increase in tail-withdrawal latencies in 50°C water; the maximum increase was observed 60 min following administration of morphine 1 mg/kg. Diltiazem 10, 20 and 40 mg/kg produced a dose-related potentiation of the magnitude of the morphine 1 mg/kg analgesic effect and increased the duration of antinociception produced by this dose of morphine (Fig. 1).

Diltiazem 40 mg/kg produced an increase of approximately 0.5 log unit in the potency of both morphine and heroin in the analgesia assay (Fig. 2). The ED_{50}s for morphine and heroin in the presence of diltiazem 40 mg/kg were 4.35 and 3.51 times lower, respectively, than the ED_{50}s in the absence of diltiazem (Table 1); diltiazem did not alter the effects of morphine and heroin at 55°C water.

Diltiazem 40 mg/kg did not modify the analgesic potencies of either fentanyl or etonitazene (Fig. 3). The dose ratios of fentanyl and etonitazene in the presence and absence of diltiazem were 1.17 and 0.92, respectively (Table 1).

Nalbuphine (0.32 to 32 mg/kg) produced an analgesic response in 50°C water in three of four monkeys. Diltiazem 40 mg/kg did not modify this analgesic response in these three monkeys (Fig. 4) and the dose ratios in the presence and absence of diltiazem were not significantly different for nalbuphine (Table 1). Diltiazem also did not modify the antinociception produced by either U-50,488 or clonidine (Fig. 5 and Table 1).

Table 2
ED_{33} values and dose ratios for different μ-opioid receptor agonist-induced respiratory suppression in the presence or absence of diltiazem (40 mg/kg)

Parameters	Agonists	Conditions	ED_{33} (mg/kg)[a]	(95% C.L.)[b]	Dose ratios[c]
Ve	Morphine	Vehicle	0.61	(0.09–4.34)	1.19
		Diltiazem	0.51	(0.16–1.67)	
	Heroin	Vehicle	0.062	(0.032–0.121)	0.87
		Diltiazem	0.071	(0.045–0.111)	
	Fentanyl	Vehicle	0.0039	(0.0005–0.0302)	0.65
		Diltiazem	0.0060	(0.0021–0.0170)	
Vt	Morphine	Vehicle	2.88	(1.23–6.73)	1.00
		Diltiazem	2.87	(0.27–30.7)	
	Heroin	Vehicle	0.44	(0.11–1.80)	0.85
		Diltiazem	0.51	(0.16–1.62)	
	Fentanyl	Vehicle	0.038	(0.025–0.059)	0.95
		Diltiazem	0.040	(0.007–0.225)	
f	Morphine	Vehicle	2.79	(0.15–53.3)	1.25
		Diltiazem	2.24	(0.40–12.5)	
	Heroin	Vehicle	0.38	(0.11–1.27)	0.62
		Diltiazem	0.61	(0.09–4.02)	
	Fentanyl	Vehicle	0.017	(0.003–0.081)	0.68
		Diltiazem	0.025	(0.005–0.131)	

[a] ED_{33} values are the mean of individual ED_{33}s, which are calculated by least_squares regression from dose_effect curves.
[b] C.L. = confidence limit.
[c] Dose ratios are calculated using corresponding ED_{33} values.

3.2. Respiration

A bolus injection of morphine 3.2 mg/kg produced a marked decrease in respiratory parameters (Fig. 6). Administration of diltiazem 40 mg/kg 30 min prior to morphine administration did not modify the respiratory depression produced by morphine, although Ve and Vt, but not *f*, were suppressed by 10–15% of control values by diltiazem alone (Fig. 6).

Cumulative administration of morphine produced a dose-dependent suppression of Ve; diltiazem did not modify the potency of morphine in this situation (Fig. 7). Heroin and fentanyl also produced dose-related decreases in respiration that were not altered by diltiazem (data not shown).

Table 2 shows the ED_{33}s of morphine, heroin, and fentanyl for the three respiratory parameters. Diltiazem did not modify the ED_{33} of morphine for *f*, Vt or Ve; the dose ratio of the morphine ED_{33} following vehicle or diltiazem was 1.25, 1.0 and 1.19 for the three parameters, respectively. Diltiazem also failed to modify the ED_{33}s for heroin or fentanyl; dose ratios for the ED_{33}s of respiration for these compounds ranged between 0.62 and 0.95.

4. Discussion

In this study, the Ca^{2+} channel blocker diltiazem, which had no analgesic effects of its own, was found to increase selectively the potency of morphine and heroin as analgesics. Diltiazem did not increase the analgesic potency of the full μ-opioid receptor agonists fentanyl and etonitazene, the partial μ-opioid receptor agonist nalbuphine, the κ-opioid receptor agonist U-50,488, or the non-opioid clonidine. Diltiazem also did not modify the potency of morphine or heroin as respiratory depressants.

Systemic administration of Ca^{2+} channel blockers has been found to potentiate morphine-induced analgesia in mice (Del Pozo et al., 1987; Contreras et al., 1988; Thirugnanasambantham et al., 1988; Del Pozo et al., 1990) and rats (Antkiewicz-Michaluk et al., 1993). In addition, central administration of L-type Ca^{2+} channel blockers has also been reported to potentiate morphine-induced antinociception in rodents (Del Pozo et al., 1990; Omote et al., 1993), indicating that L-type Ca^{2+} channel blockers interact with morphine in the central nervous system to potentiate opioid-induced analgesia. In rodents, the interaction was not limited to morphine; however, both fentanyl- and sufentanil-induced analgesia was enhanced by nimopidine in rats using vocalization and tail-flick analgesia tests (Hoffmeister and Tettenborn, 1986; Dierssen et al., 1991). On the other hand, Seyler et al. (1983) reported that another L-type Ca^{2+} channel blocker verapamil potentiated morphine analgesia but not that of methadone or propoxyphene in a mouse hot-plate assay.

Studies in humans have not been as clear cut. In studies in which morphine was being given chronically for pain, the L-type Ca^{2+} channel blocker nimodipine reduced the need to escalate the morphine dose and produced a decrease in the daily dose of morphine required (Santillan et al., 1994; 1998). Serum levels of morphine and its metabolites 3- and 6-glucuroindes were not increased (Santillan et al., 1998). Similar enhancement of morphine analgesia was reported in experimental studies in humans given both drugs acutely (Carta et al., 1990). Roca et al. (1996), however, found no enhancement of the effects of morphine by nimodipine during the initial stages of treatment of cancer pain. There is thus considerable confusion in the literature as to the nature and the specificity of the interaction between Ca^{2+} channel blockers and opioid effects. Among the variables that may be relevant, but which have not been well studied include: the species of subject; the Ca^{2+} channel blocker used; acute vs. chronic administration of the drugs; and the method of assaying analgesia. In addition, there is very little information available on the interactions observed if other effects of opioids, for example, respiratory depression, are studied.

One possible mechanism for the effects we observed here in rhesus monkeys, a diltiazem-potentiated analgesic effects of only morphine and heroin that was not observed in measures of respiratory depression, is that diltiazem is increasing the brain concentration of a metabolite that is specific to morphine and heroin. This metabolite should act centrally on a subset of central opioid receptors that mediate analgesia but not respiratory depression. One compound that may fill these requirements is morphine-6-glucuronide. Morphine-6-glucuronide is a metabolite of morphine and heroin in the rhesus monkey, but not of the other analgesics tested here (Rane et al., 1984). There is evidence using blood gas measures in humans that it does not cause respiratory depression (Thompson et al., 1995), but it is much more potent than morphine as an analgesic when it is given centrally to mice (Paul et al., 1989). If morphine-6-glucuronide concentration is increased, perhaps due to a reduced rate of exodus from the brain, even the low levels expected of a metabolite might be effective in potentiating morphine-induced analgesia. Although Santillan et al. (1998) found no increases in morphine-6-glucuronide in the serum of patients receiving combinations of morphine and nimodipine, serum levels might not reflect small but clinically important increases in central levels of this metabolite.

A possible mechanism by which diltiazem could increase retention of morphine-6-glucuronide in the central nervous system is through competition of these two compounds as substrates of an ATP-dependent drug efflux pump, P-glycoprotein. Although diltiazem has not been shown to be a substrate of the P-glycoprotein pump, other L-type Ca^{2+} channel blockers such as verapamil do have this capability (Spoelstra et al., 1994). Morphine-6-glucuronide (Huwyler et al., 1996), morphine, and other

opioids (Callaghan and Riordan, 1993) are also substrates for this pump, suggesting that an interaction among these compounds could lead to increased central levels of morphine-6-glucuronide following administration of combinations of morphine or heroin and diltiazem.

Although this hypothesis can be used to explain our data in the rhesus monkey, it does not explain why fentanyl analgesia is enhanced by L-type Ca^{2+} channel blockers in rodents, and it does not explain the conflicting results on the interaction between morphine and Ca^{2+} channel blockers in humans. Thus, considerable further testing of this hypothesis is warranted. Of particular interest would be measures of cerebrospinal fluid levels of morphine-6-glucuronide following morphine administration in the presence and absence of diltiazem administration.

These data indicate that the analgesic potency of morphine may be enhanced by co-administration of diltiazem. Diltiazem does not modify the respiratory depressant effects of morphine. This suggests that diltiazem may be an effective adjunct in the treatment of pain, particularly in situations in which the respiratory depressant side-effects of analgesic administration are of particular concern.

Acknowledgements

The authors thank Dr. Gail Winger for her assistance with the preparation of the manuscript and Z.W. Wu for his technical assistance. This research was supported by the M.H. Seevers Fellowship Fund and NIDA Grants DA00254 and DA05653. Portions of the present data were presented at the 28th meeting of the International Narcotics Research Conference in Hong Kong, China, August 3–8, 1997.

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