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Time Course of Verapamil Interaction with Morphine Effects on Physiological Parameters in Rats

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Abstract—The effects of subcutaneous doses of morphine and verapamil on respiratory and cardiovascular parameters have been assessed in conscious rats. Verapamil (10 mg kg^{-1}) was injected simultaneously with morphine (16 mg kg^{-1}) or at 10, 30, or 60 min before morphine administration. Morphine induced respiratory depression, as indicated by marked hypercapnia, hypoxia and acidosis, and caused marked tachycardia. Although morphine produced only a minor and inconsistent (but statistically significant, $P < 0.01$) reduction of mean arterial blood pressure, morphine potentiated verapamil-induced hypotension. Verapamil suppressed morphine-induced hypercapnia only when injected simultaneously with morphine. Verapamil alone did not affect arterial blood gases or pH, but decreased heart rate and mean arterial blood pressure. Verapamil attenuated and delayed the maximum positive chronotropic effects of morphine at all times tested. Antagonism by verapamil of respiratory depression and tachycardia produced by morphine was unrelated to morphine levels in plasma. Thus, the explanation of verapamil-morphine interactions on respiration and cardiovascular function is not pharmacokinetic.

Calcium channel antagonists, such as verapamil and diltiazem, are used clinically in cardiovascular therapy (Henry 1980; Baky & Singh 1982; Wagniart et al 1982). Studies of opioid analgesia, tolerance and dependence in rats have used drugs that inhibit calcium binding and transport. One such study has shown that intracerebral injection of lanthanum ion produces analgesia, an effect which varies with opioid tolerance and dependence (Harris et al 1975). In addition, both verapamil and diltiazem facilitate morphine analgesia and hypothermia (Benedek & Szikszay 1984); however, these drugs delay and/or antagonize indices of morphine-induced respiratory depression (Szikszay et al 1986a,b). Hence, calcium channel blockers can have opposite effects on different responses to opioids. The present study was an extension of earlier observations on the interactions of verapamil with morphine-induced respiratory depression and cardiovascular effects (Szikszay et al 1986b). The aim was to explore potential pharmacokinetic contributions to the interactions between morphine and verapamil by using time course and drug concentration determinations.

Materials and Methods

Preparation of animals

Male Fischer-344 rats (n = 64) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA), at 3 to 4 months of age. On the day of experimentation, 2% halothane was administered to achieve anaesthesia (Fluothec Mark II vaporizer, Ohmeda, Inc.), and a polyethylene catheter was inserted into the left femoral artery. The wound was closed with sutures, and 0·3 mL of bupivacaine 5% infiltrated. The animals were partially restrained (London et al 1981) and were allowed to recover from anaesthesia for approximately 3 h before drug treatment. A rectal thermistor probe was inserted to monitor body temperature, which was maintained at 37°C by external heating.

Drugs

The drugs used were morphine sulphate (Mallinckrodt, Inc., St. Louis, MO) and verapamil HCl (Sigma Chemical Co., St. Louis, MO), each dissolved in 0·9% NaCl (saline). The following four treatments were administered: saline/saline; saline/morphine; verapamil/saline and verapamil/morphine. In each treatment group, verapamil or saline was injected at 0, 10, 30 or 60 min before the second injection, which contained either morphine or saline. Verapamil and morphine were given at doses of 10 mg kg^{-1} and 16 mg kg^{-1}, respectively, calculated as the salts. The selected dose of verapamil antagonizes respiratory depression when simultaneously administered with 16 mg kg^{-1} of morphine (Szikszay et al 1986b). Verapamil and morphine were injected subcutaneously, each in a volume of 1 mL kg^{-1} body weight, while control animals received the same volume of 0·9% NaCl.

Physiological measurements

Blood pressure and heart rate were monitored by connecting the arterial catheter to a strain gauge transducer (Gould Statham Instruments, Inc., Hatorey, PR), connected to a paper chart recorder (Model 2200, Gould Instruments, Cleveland, OH). Physiological parameters were measured and arterial blood samples were withdrawn 5 min before the first injection (baseline) and at the following times after the second injection: 15, 30, 60 and 120 min. Arterial blood Pa_{CO_2}, Pa_{O_2} and pH were determined using a pH-Blood Gas Analyzer (System 1302, Instrumentation Laboratory, Inc., Lexington, MA).

Morphine determinations

Free morphine levels in plasma were determined by radioimmunoassay utilizing an antibody (Coat-A-Count Serum Morphine, Diagnostic Products Corp., Los Angeles, CA)

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that demonstrates minimal cross-reactivity with morphine-3-glucuronide (0·025%) and normorphine (2·66%). Specimens were assayed for free morphine on the basis of a morphine standard curve prepared in a rat plasma matrix. Serial dilutions of plasma were prepared to obtain levels within the range of the standard curve (0–250 ng mL^{-1}). The accuracy of the assay was monitored by inclusion of morphine controls. Overall mean determinations ± s.e. for 15·0 and 50·0 ng mL^{-1} morphine controls (n = 30) were 14·8 ± 0·5 and 46·7 ± 1·2 ng mL^{-1}, respectively. Within run and between run coefficients of variation for the assay were 11·8 and 13·2%, respectively.

Statistical analysis

To assess the effects of morphine and verapamil, alone and in combination, and the time course of these effects, a four-factor analysis of covariance was performed on the physiological measures. Analysis of covariance was used to account for individual differences in baseline values. The main effects tested were time of verapamil injection (relative to morphine), verapamil (vs saline), morphine (vs saline) and time as a repeated measure. The criterion for significance statements was $P < 0·05$. Effects of verapamil on the time of peak effect of morphine (determined separately for each animal) were also tested separately by a two-factor analysis of variance, with time of verapamil (or saline) injection and verapamil treatment as the factors. Only those treatment groups which received morphine (with or without verapamil) were included in this analysis. In cases where a significant interaction was obtained, Tukey's ω procedure (Keppel 1980) was used to analyse the nature of the interaction.

The effect of drug treatments on morphine plasma concentrations was assessed by a one-way analysis of variance of individual rat morphine area under the curve (AUC) measurements (0–120 min). AUC calculations were made using the trapezoidal rule.

Results

Respiratory parameters

Verapamil, when administered alone, did not affect arterial Pa_{CO_2} or Pa_{O_2} whereas morphine injected without verapamil, depressed respiration, producing hypercapnia and hypoxia peak effects at 30 min–1 h after administration (Table 1, Figs 1, 2). When injected simultaneously with morphine, verapamil significantly reduced the morphine-induced hypercapnia and hypoxia, but did not alter these effects of morphine when given 10, 30 or 60 min before morphine (Figs 1, 2). The duration of the action of verapamil on morphine-induced hypercapnia was approximately 60 min, while the antagonism of hypoxia had a duration of 30–60 min. Although the antagonistic properties of verapamil on morphine-induced hypercapnia were attenuated by increasing the time between the verapamil injection and the later injection of morphine (Fig. 1), verapamil delayed the onset of the hypercapnic peak produced by morphine. Analysis of the time of peak morphine effects determined separately for each animal (data not shown) showed the delay to be statistically significant in every group that received verapamil and morphine.

Administration of verapamil or saline, without morphine, did not affect arterial pH during the experiment. However, verapamil increased morphine-induced acidosis when administered simultaneously with morphine or up to 60 min before the morphine injection (Fig. 3).

Cardiovascular parameters

When administered alone, morphine produced marked tachycardia with the maximum effect at 30 to 120 min after the drug injection (Table 1, Fig. 4). Verapamil blocked morphine-induced tachycardia, irrespective of the verapamil injection time, as demonstrated by the significant time × verapamil × morphine interaction. The onset of the antagonism

Table 1. Four-way analysis of covariance showing main effects and interaction of the following factors on cardiovascular and respiratory parameters: morphine (M), verapamil (V), verapamil injection time (I), and time after morphine injection (T). N = 4 per group. Tabulated numbers are values of F for the dF indicated in parentheses.

	Parameters measured					
	Mean blood pressure	Heart rate		Arterial blood Pa_{O_2}	Pa_{CO_2}	pH
Main effects			Main effects			
I (3,45)	1·39	2·31	I (3, 46)	0·73	0·18	0·92
V (1, 45)	151·92***	6·43*	V (1, 46)	3·41	1·39	2·70
M (1, 45)	9·33**	26·92***	M (1, 46)	60·12***	202·52***	263·05***
T (3, 138)	5·19**	4·95**	T (3, 141)	4·23**	5·34**	41·81***
Interactions			Interactions			
I × V (3, 45)	1·19	0·21	I × V (3, 46)	1·13	4·62**	1·20
I × M (3, 45)	0·68	0·22	I × M (3, 46)	0·20	0·57	0·60
V × M (1, 45)	7·16*	15·67***	V × M (1, 46)	0·14	1·32	0·08
I × V × M (3, 45)	0·29	0·33	I × V × M (3, 46)	0·24	0·38	0·30
T × I (9, 138)	0·86	0·77	T × I (9, 141)	1·62	0·77	1·46
T × V (3, 138)	5·54**	6·19***	T × V (3, 141)	2·42	3·51**	4·73**
T × M (3, 138)	9·98***	2·65*	T × M (3, 141)	4·76**	11·06***	40·90***
T × I × V (9, 138)	0·48	0·90	T × I × V (9, 141)	1·02	0·85	0·51
T × I × M (9, 138)	1·01	1·21	T × I × M (9, 141)	1·20	0·93	0·69
T × V × M (3, 138)	9·63***	5·18**	T × V × M (3, 141)	0·78	1·46	2·15
T × I × V × M (9, 138)	1·22	1·11	T × I × V × M (9, 141)	0·51	0·49	1·79

*Significant at $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

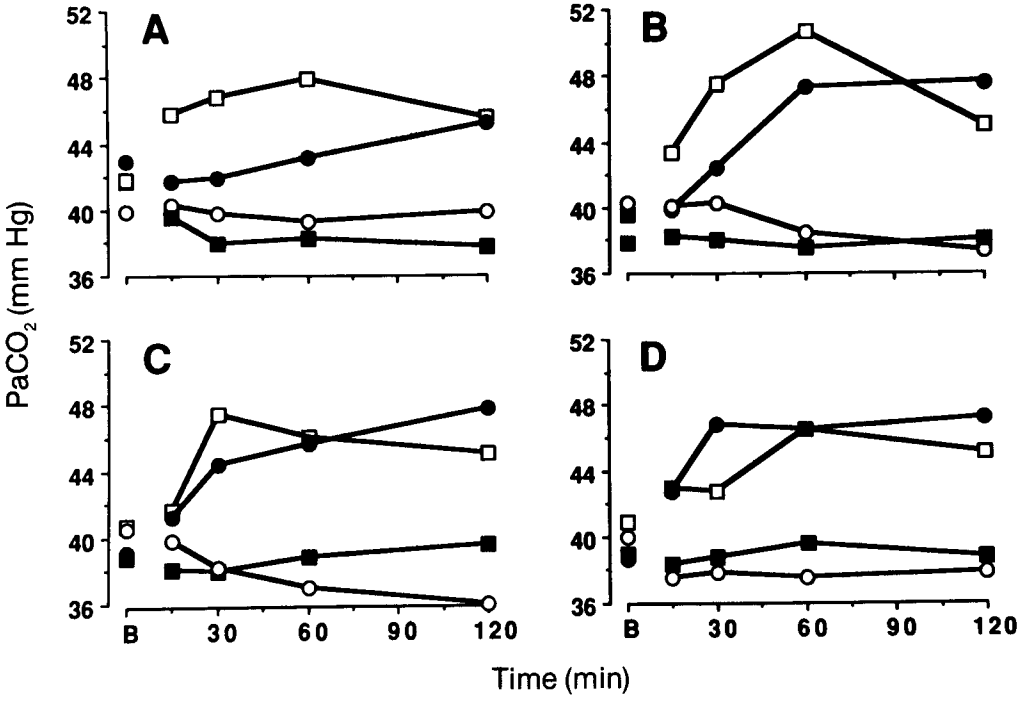

FIG. 1. Effects of verapamil pretreatment and morphine on Pa_{CO_2}. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show Pa_{CO_2} (mm Hg) in different treatments tested: (○) saline/saline; (■) verapamil/saline, (□) saline/morphine, and (●) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group. See Table 1 for statistical significance.

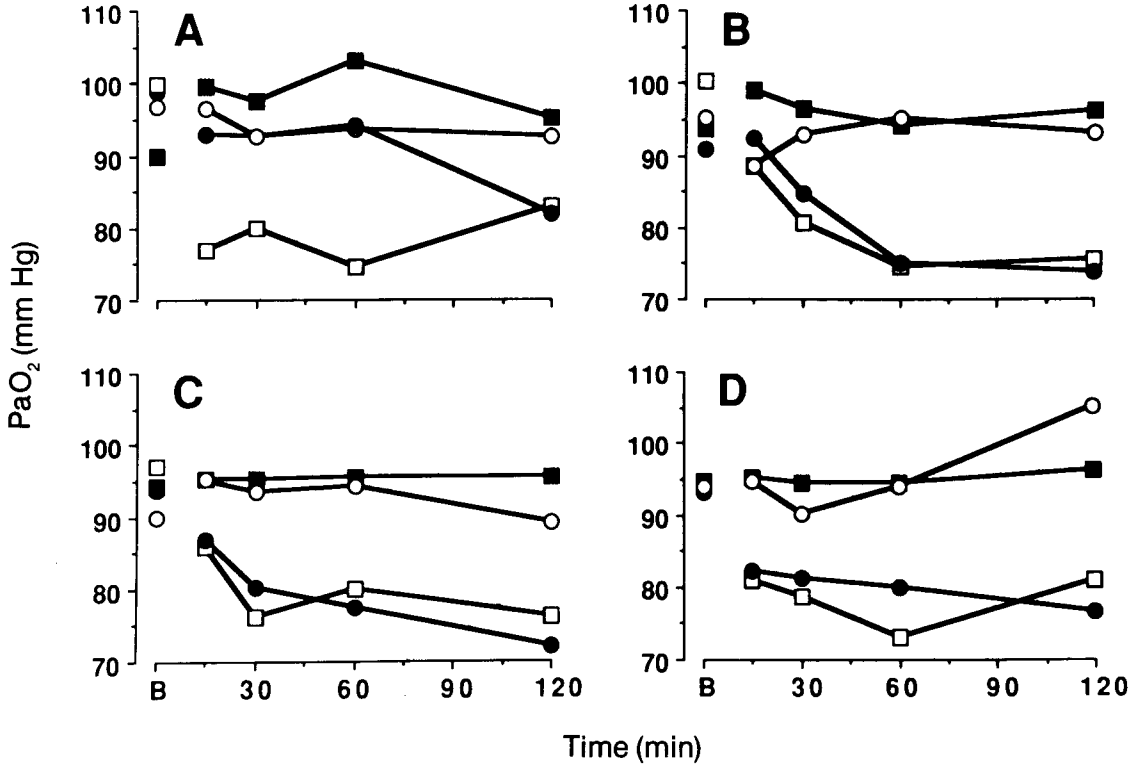

FIG. 2. Effects of verapamil pretreatment and morphine on arterial blood Pa_{O_2}. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show Pa_{O_2} (mm Hg) in different treatments tested: (○) saline/saline; (■) verapamil/saline, (□) saline/morphine, and (●) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group. See Table 1 for statistical significance.

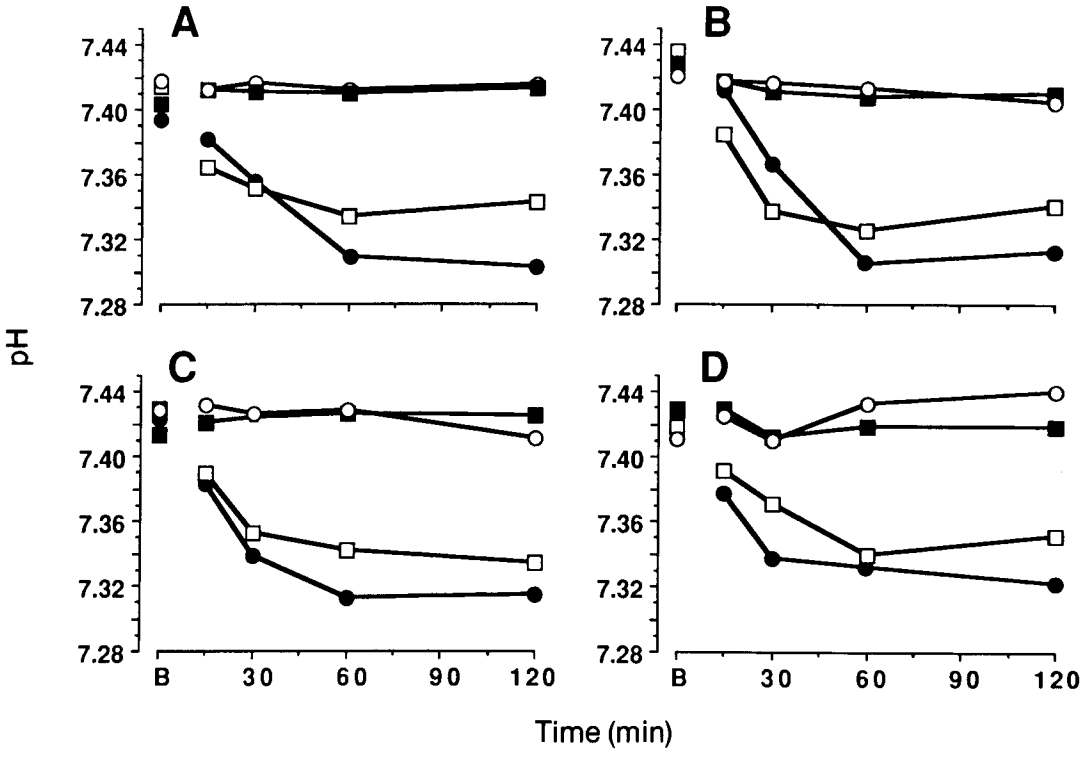

FIG. 3. Effects of verapamil pretreatment and morphine on arterial blood pH. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show pH in different treatments tested: (○) saline/saline; (■) verapamil/saline, (□) saline/morphine, and (●) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group. See Table 1 for statistical significance.

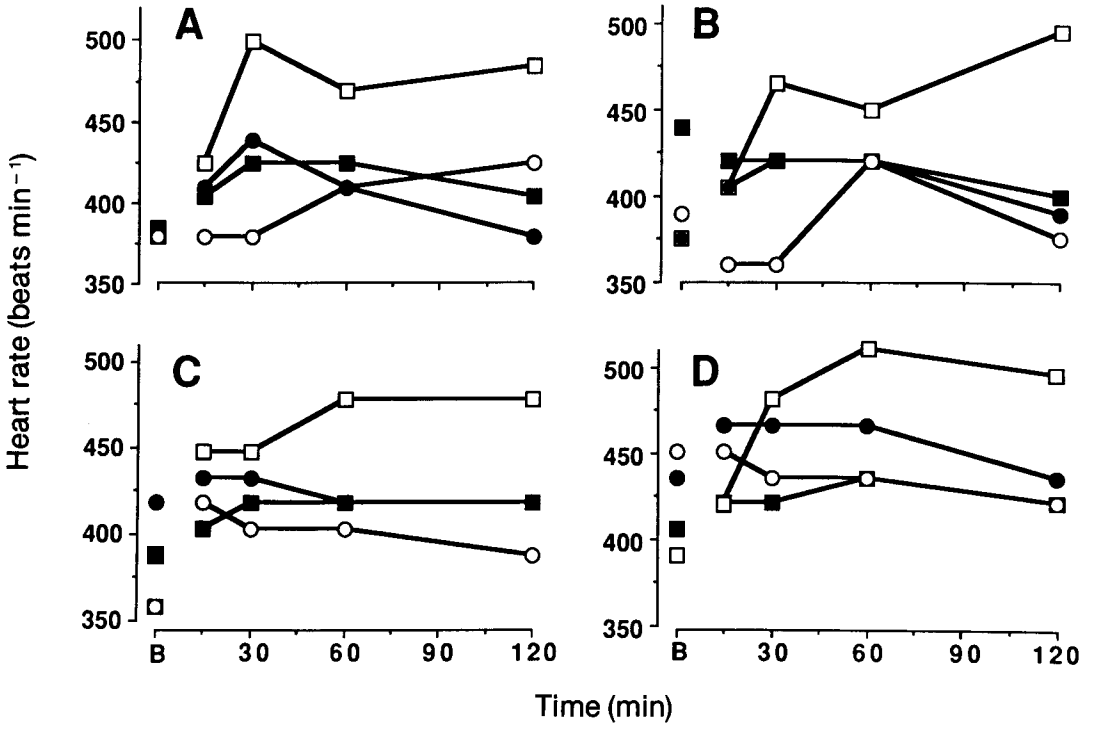

FIG. 4. Effects of verapamil pretreatment and morphine on heart rate. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show heart rate, HR (beat/min) in different treatments tested: (○) saline/saline; (■) verapamil/saline, (□) saline/morphine, and (●) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group. See Table 1 for statistical significance.

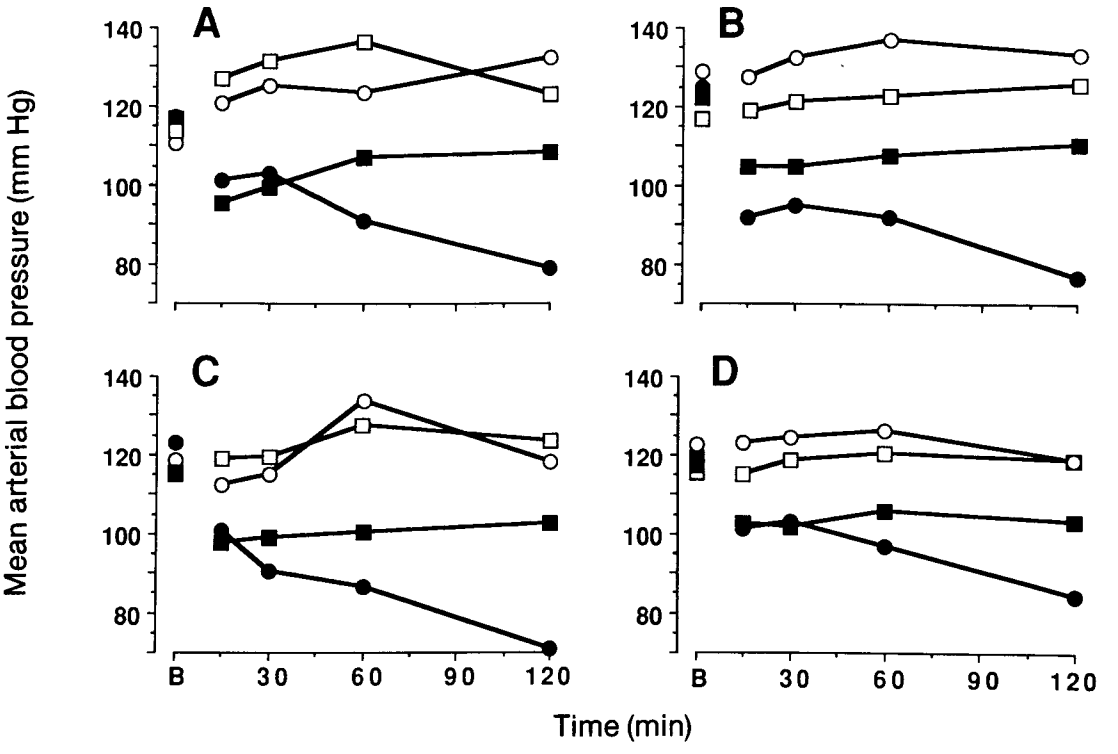

FIG. 5. Effects of verapamil pretreatment and morphine on mean arterial blood pressure. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show mean arterial blood pressure, MBP (mm Hg) in different treatments tested: (○) saline/saline; (■) verapamil/saline, (□) saline/morphine, and (●) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group. See Table 1 for statistical significance.

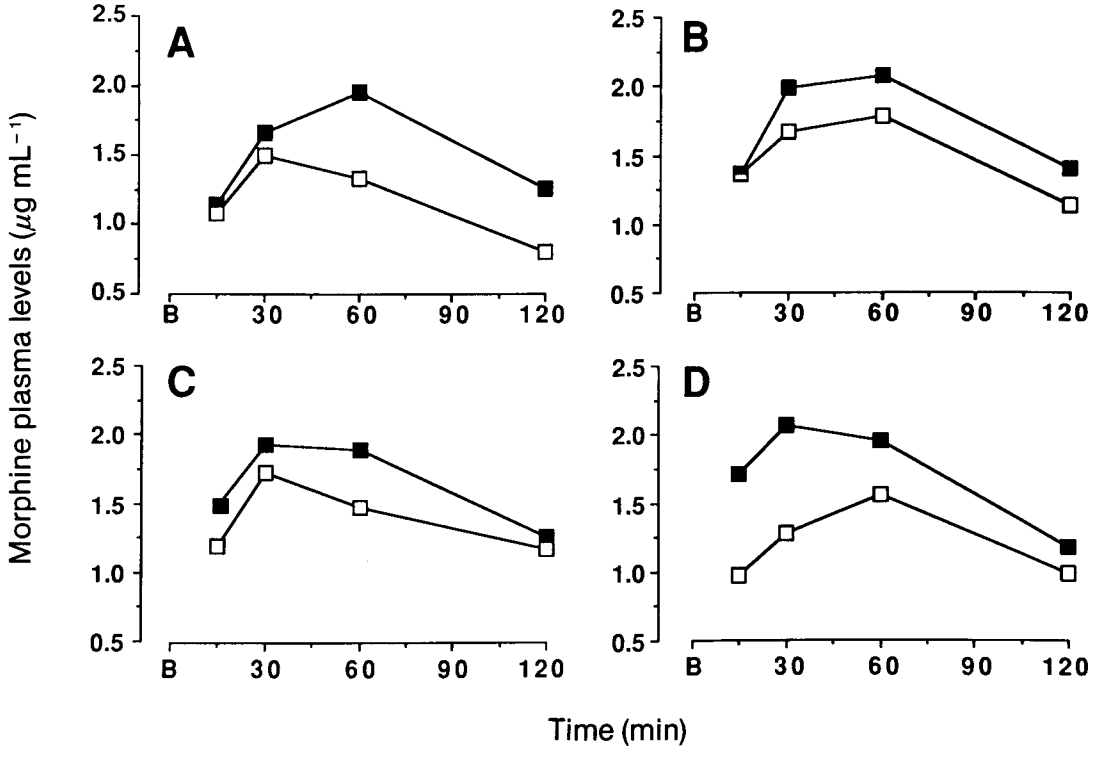

FIG. 6. Effects of verapamil pretreatment on morphine levels in plasma. Abscissae show the sampling time after morphine injection, with B representing baseline values. Ordinates show morphine plasma levels ($\mu g\ mL^{-1}$) in different treatments tested: (□) saline/morphine, and (■) verapamil/morphine. Verapamil or saline were given simultaneously with morphine (A), or 10 min (B), 30 min (C), 60 min (D), before morphine. Each point represents the mean obtained from four rats per group.

was evident at the first time of sampling (15 min after morphine), with maximum inhibition occurring at 2 to 3 h after the verapamil injection. Verapamil and morphine given independently of one another both significantly decreased mean arterial blood pressure. The effect of verapamil was evident throughout the experimental period, and was more substantial than that of morphine, which was minor and inconsistent (Table 1, Fig. 5). However, morphine facilitated the hypotensive effect of verapamil. The potentiation did not depend on the verapamil injection time. Peak hypotension in most groups tested appeared at 15 min after verapamil administration, with subsequent increases in blood pressure toward baseline in the verapamil/saline groups. Hypotension progressed with increasing time in the verapamil/morphine groups, and was most severe at the 120 min time point.

Concentrations of morphine in plasma

In groups receiving morphine only, mean morphine levels in plasma at 15 min after the morphine injection ranged from 0·97 to 1·37 $\mu g\ mL^{-1}$ (Fig. 6). Corresponding values in the verapamil/morphine groups ranged from 1·14 to 1·71 $\mu g\ mL^{-1}$. Thereafter, morphine levels in all drug treatment groups generally rose slightly, reaching the highest levels at 30–60 min, and falling slightly by 120 min after morphine administration. Although morphine levels were higher after verapamil/morphine vs saline/morphine treatment, this effect was not statistically significant.

Discussion

In agreement with previous work showing a dilator action of calcium channel antagonists on coronary and peripheral arteries (Haeusler 1972; Zsoter & Church 1983), verapamil, in the present study, produced marked hypotension, which was facilitated by morphine. Several possible factors could account for the interaction. Verapamil might manifest the peripheral vasodilative effect of morphine by counteracting the morphine-induced cardiac stimulation. Furthermore, the acidosis observed in the verapamil/morphine groups could facilitate the vasodilative action of verapamil. In support of this view, maximum hypotension in the verapamil/morphine groups generally coincided with the greatest facilitation by verapamil of morphine-induced, acidosis and blockade by verapamil of morphine-induced tachycardia. The positive interaction between morphine and verapamil on hypotension was unrelated to plasma morphine levels, as the time that showed the greatest interaction was 120 min after morphine treatment, when morphine levels in all treatment groups were at the lowest levels assayed. The interactions between verapamil and morphine on blood pressure suggest that combined treatments with calcium channel antagonists and opioids may produce untoward effects in clinical situations.

In mammals, variations in Pa_{CO_2} directly affect the concentration of carbonic acid in the blood. Since the buffering properties of the HCO_3^- and the H^+ H_2 CO_3 system in the blood are not strong, the acidotic effects of morphine usually reflect morphine-induced hypercapnia. The present results confirm morphine-induced acidosis, which is facilitated by verapamil (Szikszay et al 1986b) and it seems that the enhanced acidosis may reflect the cardiovascular effects of verapamil. Reduction in cardiac chronotropism and inotropism by calcium channel blockade (Needleman et al 1985), along with hypercapnia, hypoxia and hypotension, may have reduced peripheral circulation, and enhanced metabolic acidosis in the verapamil/morphine groups.

Verapamil effectively reduced morphine-induced hypoxia and hypercapnia when given simultaneously with morphine. This antagonistic effect of verapamil was unrelated to morphine levels in the plasma, as the greatest antagonism of morphine-induced respiratory depression occurred when levels of morphine were most similar in groups with and without verapamil treatment. Although the mechanism of the antagonism of opioid respiratory depressant effects by verapamil is not known, it is established that opioids decrease synaptosomal calcium content and uptake (Ross & Cardenas 1979; Chapman & Way 1980). If these actions on calcium are important to morphine-induced respiratory depression, verapamil might antagonize the respiratory effects by blockade of calcium channels.

Morphine has biphasic effects on heart rate and blood pressure in cats, with low doses decreasing and high doses increasing blood pressure and heart rate (Wallenstein 1979). Furthermore, 4–16 mg kg^{-1} morphine (s.c.) increases heart rate in partially immobilized, conscious rats (Szikszay et al 1986b). Verapamil generally did not reduce heart rate but antagonized morphine-induced tachycardia. The antagonism was not related to a reduced bioavailability of morphine, as morphine levels in plasma from verapamil-treated rats were either the same or higher than those in animals that did not receive verapamil. Perhaps the effect of verapamil on heart rate was more evident in the morphinized rats, which showed tachycardia, because the effect of calcium channel blockade on atrioventricular conduction varies with the frequency of stimulation (Needleman et al 1985).

Although the tendency of verapamil to increase the concentration of morphine in arterial plasma did not reach statistical significance, the trend was consistent with the ability of verapamil to impair the metabolism of a variety of drugs in laboratory animals and humans (Renton 1985, Edwards et al 1987) and to decrease hepatic blood flow and systemic drug clearance (Hamann et al 1984).

The present findings indicate that the nature of verapamil-morphine interactions is complex. While verapamil briefly antagonizes morphine-induced respiratory depression, it reduces morphine-induced tachycardia for a relatively long time. Verapamil also facilitates morphine-induced acidosis while morphine facilitates the hypotensive effect of verapamil. The interactions do not reflect an alteration in morphine pharmacokinetics. Hence, they appear to reflect actions of verapamil on other biochemical or physiological substrates through which the physiological effects of morphine are mediated.

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