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Effects of diltiazem on morphine-induced respiratory decline

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The individual and combined effects of subcutaneous morphine and diltiazem, a calcium channel inhibitor, on arterial blood gases and pH were assessed in conscious Fischer-344 rats. Morphine (4 mg kg^{-1}) produced hypercapnia, hypoxia and slight acidosis, as compared with control values. Diltiazem (10 mg kg^{-1}) alone did not affect these parameters; however, it significantly delayed the onset of the aforementioned effects of morphine.

Diltiazem is a calcium channel inhibitor, which has been used clinically to treat coronary heart disease, hypertension and arrhythmias (Henry 1980). Calcium channel inhibitors specifically reduce Ca^{2+} influx through voltage-dependent calcium channels (for reviews see Chaffman & Brogden 1985; Schramm & Towart 1985). Opioids can inhibit Ca^{2+} uptake and influx in a naloxone-reversible manner (for reviews see Ross & Cardenas 1979; Chapman & Way 1980). Whereas local Ca^{2+} injections into the brain antagonize opioid-induced analgesia in rodents (Hano et al 1964; Guerrero-Munoz et al 1981), calcium channel inhibitors can potentiate the antinociceptive (Konno & Takayanagi 1983; Ben-Sreti et al 1983) and simultaneous thermoregulatory effects of opioids (Benedek & Szikszay 1984).

Several studies have suggested that opioids produce analgesia and respiratory depression through different mechanisms (McGillard & Takemori 1978; Ling et al 1983, 1985). It was of interest to investigate whether a calcium channel inhibitor might differentially affect morphine-induced analgesia, hypothermia and respiratory depression. In the light of previous findings that diltiazem potentiates morphine-induced antinociception and hypothermia in rats (Benedek & Szikszay 1984), the objective of this work was to investigate the interaction between morphine and diltiazem on morphine-induced respiratory decline.

Materials and methods

Twenty-four male, 3–4 month old Fischer-344 rats were prepared with a femoral arterial catheter under halothane anaesthesia and were partially immobilized as described previously (London et al 1981). After 3 h, arterial blood samples were taken from conscious rats 5 min before drug treatments and at 0·25, 0·5, 1 and 2 h after injections for measuring blood gases and pH.

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Drug treatments. Morphine sulphate (Merck & Co., Inc., Rahway, NJ) and diltiazem hydrochloride (Marion Laboratories, Inc., Kansas City, MO) were dissolved in 0·9% NaCl. The dosages of morphine sulphate and diltiazem were 4 and 10 mg kg^{-1}, respectively, expressed as the salts. The drugs were injected s.c. either alone or in combination. Control rats received saline.

Statistical analysis. Effects of drug treatments at different times were assessed by a two-way analysis of variance (ANOVA). Post-hoc comparisons were performed using Tukey's ω-procedure. A one-way ANOVA was performed on both the magnitudes and times of the peak effects for every measured parameter, and morphine vs morphine + diltiazem effects were compared using Bonferroni t statistics (Keppel 1980). The criterion for all significance statements was $P \leq 0{\cdot}05$.

Results

Baseline values of arterial blood P_aO_2, P_aCO_2 and pH ranged from 75–93, 31–40, 7·382–7·466 mm Hg, respectively. The two-way ANOVA demonstrated significant main effects for drugs and time and the drug by time interaction for all measured parameters; $F(12, 80) = 10{\cdot}9, 4{\cdot}6, 8{\cdot}5$, respectively. As can be seen in Fig. 1, diltiazem produced no changes in blood gases or pH relative to control, but morphine, either alone or in combination with diltiazem, produced a time-dependent hypoxia, hypercapnia and acidosis. However, rats treated with the morphine + diltiazem combination had less severe hypoxia, hypercapnia and acidosis at 15 min than those given morphine alone.

The mean magnitudes and times of the peak effects, as determined using the data from each rat, are shown in Table 1. Diltiazem significantly delayed the onset of the maximum hypercapnia and hypoxia induced by morphine. The effects of this relatively small dose of morphine, with or without diltiazem, on blood gases and pH were of similar magnitude.

Discussion

An interaction between morphine and the calcium ion has been well established. Moreover, calcium channel inhibitors (aspaminol, gallopamil) potentiate analgesia induced by morphine (Konno & Takayanagi 1983) and 6,7-benzomorphans (Ben-Sreti et al 1983). In contrast with the potentiation between morphine and diltiazem

Table 1. Magnitudes and times of peak respiratory depressant effects of morphine and diltiazem (mean ± s.e. of 6 rats).

	Magnitude of peak effect		Time of peak effect (h)	
	Morphine	Morphine + diltiazem	Morphine	Morphine + diltiazem
ΔP_aO_2 (mm Hg)	−17·67 ± 2·26	−15·67 ± 2·28	0·46 ± 0·04	0·92 ± 0·08*
ΔP_aCO_2 (mm Hg)	5·07 ± 0·84	4·10 ± 0·94	0·38 ± 0·06	1·00 ± 0·23*
Δ pH	−0·083 ± 0·007	−0·084 ± 0·007	0·75 ± 0·11	1·00 ± 0·00

[a] Each number represents the mean of 6 values, each being the time measured (0·25, 0·5, 1 or 2 h) when a rat showed the maximum difference from baseline.

* Significantly different from morphine treated rats, $P \leq 0{\cdot}05$ (Bonferroni t statistics).

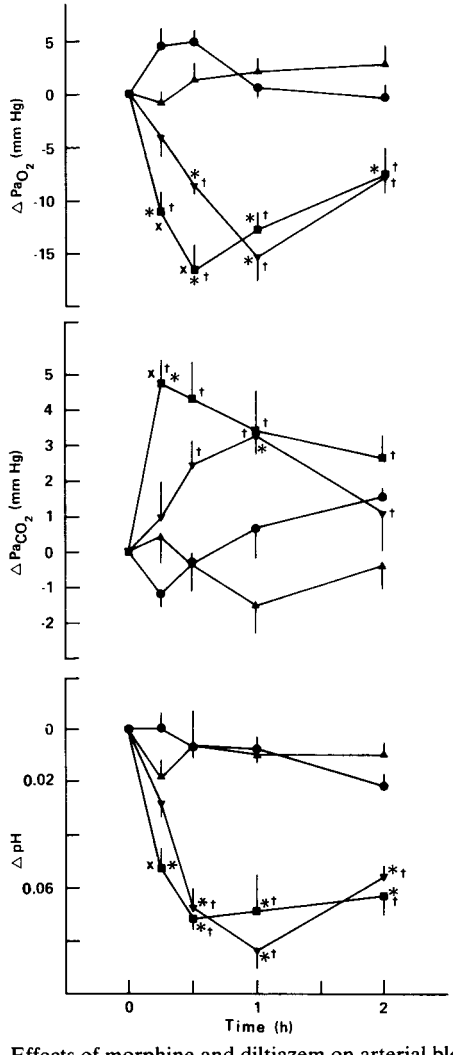

FIG. 1. Effects of morphine and diltiazem on arterial blood gases and pH. Arterial blood was sampled before (0 time) and at four times after the s.c. injection of 0·9% NaCl (▲—▲), 4 mg kg^{-1} morphine (■—■), 10 mg kg^{-1} diltiazem (●—●), or 4 mg kg^{-1} morphine + 10 mg kg^{-1} diltiazem (▼—▼). Each point represents the mean deviation from baseline values ± s.e. for 6 rats. * Significantly different from baseline, $P \leq 0{\cdot}05$. † Significantly different from control, $P \leq 0{\cdot}05$. × Significantly different from each other (morphine vs morphine + diltiazem) at $P \leq 0{\cdot}05$.

or verapamil with regard to antinociception and thermoregulation (Benedek & Szikszay 1984), the patterns of interaction here represent a delay in the appearance of morphine-induced respiratory decline.

It is well-known that opioids produce respiratory depression (Jaffe & Martin 1980). The effects of morphine on respiration have been manifested as a decline in respiratory rate (McGillard & Takemori 1978), with decreases in P_aO_2 and pH, and an increase in P_aCO_2 of arterial blood (Miller et al 1972; Ling et al 1983, 1985). These effects were also observed in the present study. Whereas diltiazem alone did not affect these parameters, it significantly delayed morphine's effects on indices of respiratory decline.

The present findings, showing an increased latency to the peak effects of morphine on indices of respiratory function, despite the reported potentiation of analgesia and hypothermia (Benedek & Szikszay 1984), agree with the classification by Pasternak et al (1983). They suggested that different opioid receptor subtypes mediate the various opioid effects (i.e. the respiratory depressant actions of opiates are mediated by different receptor sub-populations than the analgesic and thermoregulatory effects). The present physiological findings further suggest a differential interaction of Ca^{2+} with such receptor subtypes.

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Laxatives and the production of autacoids by rat colon

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The effects of some laxatives were examined on the formation of histamine, 5-hydroxytryptamine (5-HT) and prostaglandin-like material (PG-LM) by rat intestine in-vitro. Castor oil, senna, sulphosuccinate and bisacodyl, but not mannitol or lactulose, in doses that cause laxation, increased the formation of histamine, 5-HT and PG-LM. Indomethacin or hydrocortisone reduced the increase of PG-LM formation. The data support the idea that the laxative effects of these intestinal secretagogues are due to increased intestinal production of PG-LM, histamine and 5-HT.

Prostaglandins (PGs) inhibit absorption of water and electrolytes in the gut and consequently increase intestinal fluid volume (Pierce et al 1971). Such effects are similar in many respects to those caused by intestinal secretagogues (Beubler & Juan 1979). Histamine and 5-hydroxytryptamine (5-HT) also stimulate intestinal fluid and ion secretion (Koskowski 1926; Lee & Silverberg 1976; Donowitz et al 1977), and we have recently shown that phenolphthalein increases the formation of histamine, 5-HT and prostaglandin-like material (PG-LM) by rat intestine (Autore et al 1984). Our findings support the hypothesis that these three substances contribute to the laxative effect of phenolphthalein. In this paper we present evidence that other luminal secretagogues stimulate the output of histamine, 5-HT and PG-LM by rat colon.

Materials and methods

Male, Wistar Nossan rats (Correzzana, Italy), 150–160 g, were deprived of food overnight but allowed free access to water. Bisacodyl (5 mg kg^{-1}), castor oil (2 ml/rat), lactulose (5 g kg^{-1}), mannitol (10 g kg^{-1}), senna (50 mg kg^{-1}), sulphosuccinate (20 mg kg^{-1}) and water (control, 1 ml/rat) were administered by gavage. When diarrhoea was evident, the rats were killed by exposure to ether and bled. Specimens of colon were removed, rinsed in 150 mM NaCl and immediately weighed. For extraction of 5-HT, 1 M HCl was added (w/v 1:2), the tissue cut finely with scissors, homogenized, boiled for 1–2 min and centrifuged at 5000*g* for 10 min. Supernatants were neutralized with 1 M NaOH and assayed on rat gastric fundus strips in 5 ml Krebs-Henseleit solution bubbled with 5% CO_2 in O_2 at 37 °C. The bathing fluid contained (μg ml^{-1}) atropine 0·1, propranolol 0·2, mepyramine 0·1 and phenoxybenzamine 0·5. The specificity of the assay for 5-HT was checked with methysergide 0·1 μg ml^{-1}. Histamine was extracted by the same procedure except that the pH of the boiled solution was 2. The extract was bioassayed on the guinea-pig ileum in 5 ml Tyrode solution bubbled with 5% CO_2 in O_2 at 37 °C. The bathing fluid contained atropine, methysergide, propranolol and phenoxybenzamine in the concentrations used above, and the specificity of the histamine assay was checked with mepyramine 0·1 μg ml^{-1}. For extraction of PG-LM, ethanol was added to the finely cut tissue, decanted and evaporated to dryness under N_2. The tissue was then homogenized in ethyl acetate: Krebs solution buffered with Sörensen's citrate/HCl 0·1 M solution (1:1), final pH 3·0 (5:2·5). The dry extract was dissolved in 1 ml Krebs solution and bioassayed on rat gastric fundus strips in the presence of atropine, mepyramine, methysergide, propranolol, phenoxybenzamine (in the concentrations used above) and indomethacin (0·2 μg ml^{-1}). Some experiments were performed on rats pretreated with indomethacin (4 mg kg^{-1}) or hydrocortisone (20 mg kg^{-1}) injected, 48, 24 and 12 h before starting the experiment.

The following drugs were used: bisacodyl, dioctyl sulphosuccinate, mannitol, lactulose, hydrocortisone, histamine, 5-HT (all from Sigma); indomethacin (Gianni); PGE_2 (Upjohn); castor oil (Carlo Erba); senna (Senade: Andard mount). All other chemicals

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