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Actions of Opiate Agonists, Naloxone, and Paraben Preservatives in the Rat Lung Circulation[1] (42272)

SHIH-WEN CHANG AND NORBERT F. VOELKEL

Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262

Abstract. Previous studies have documented direct vascular effects of opiate substances in the systemic circulation. Because opiate receptors have been identified in the lung, we wondered whether opiate substances might affect vasoreactivity in the lung circulation. We studied the pulmonary vascular effects of three opiate agonists: morphine, leucine-enkephalin, and dynorphin, as well as the opiate receptor antagonist naloxone, in isolated rat lungs perfused with a cell- and plasma-free salt solution. Because of previous reports of the smooth muscle effects of the methyl- and propylparaben preservatives in the naloxone preparation, we also studied the pulmonary vascular effects of these preservatives in the rat lung circulation. We found that morphine, a μ-receptor agonist, leucine-enkephalin, a δ-receptor agonist, and dynorphin, a κ-receptor agonist, caused no immediate vascular effect when injected into the pulmonary artery. In addition, morphine did not affect the pulmonary vasoconstrictions induced by hypoxia, angiotensin II, or potassium chloride. The commercial preparation of naloxone, Narcan, caused a marked vasodilation during hypoxic pulmonary vasoconstriction. However, this effect was entirely attributable to the preservatives methyl- and propylparaben, as pure naloxone had no effect on either the baseline pulmonary vascular tone or the vasoconstrictive response to hypoxia. We conclude that opiate receptor agonists and antagonists do not affect vasoreactivity in the rat lung circulation and that the methyl- and propylparaben preservatives in Narcan are pulmonary vasodilators.

Since the discovery of endogenous opioid systems, opiate-like substances as well as opiate receptors have been identified in various extraneuronal tissues including the lung (1, 2). Previous studies have shown dose-related actions of opiates on various vascular systems including the rat aorta (3, 4), rat mesenteric arterioles and venules (5), rabbit ear artery (6), and cat middle cerebral artery (7). On the basis of these studies, Altura *et al.* have proposed the existence of opiate receptors in the microcirculation (5). Despite these reports of opiate actions on the systemic circulation, very little is known regarding the direct effects of opiates on the pulmonary circulation.

To answer the question whether opiate substances affect vasoreactivity in the lung circulation, we chose to study in isolated rat lungs the vascular effects of agonists to the μ, δ, and κ opiate receptors and of the receptor antagonist, naloxone. Morphine sulfate, leucine-enkephalin, dynorphin fragment, and naloxone were injected during baseline perfusion to quantitate any immediate vascular effects. Because the low vascular tone of the pulmonary circulation under baseline conditions may mask a vasodilatory action of these agents, we also examined the effects of morphine sulfate during the vasoconstrictions induced by hypoxia and potassium chloride. Because naloxone has been reported to attenuate hypoxic pulmonary vasoconstriction (8), and because of reports that the vascular action of naloxone on the cerebral vessels was attributable to the preservatives methyl- and propylparaben (9, 10), we assessed the pulmonary vascular effects of Narcan, pure naloxone, methylparaben, and propylparaben during hypoxic vasoconstriction in isolated rat lungs.

Materials and Methods. *Isolated rat lung preparations.* Male Sprague–Dawley rats (240–450 g) were anesthetized with pentobarbital sodium (10 mg/100 g ip), injected with heparin 100 IU intraventricularly, and the lungs were isolated according to techniques described previously (11, 12). To study the direct effects of the drugs on the pulmonary circulation

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without the presence of blood cells, the lungs were perfused with a physiological salt solution (PSS) containing (in m*M*): 119 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 22.6 $NaHCO_3$, 1.18 KH_2PO_4, 1.6 $CaCl_2$, 5.5 glucose, and 50 sucrose. The solution also contained 4 g Ficoll (mol wt 70,000, Sigma Co., St. Louis, Mo.) in an attempt to normalize the perfusate oncotic pressure. After the lungs were isolated, the first 50 ml of the PSS-Ficoll perfusate were discarded, leaving a cell- and plasma-free recirculating perfusate of 50-ml vol. During subsequent perfusion at constant flow (0.03 ml/g rat body wt/min), the pulmonary arterial inflow pressure was measured with a Statham transducer and continuously recorded on a Soltec recorder. The lungs were ventilated with a gas mixture containing 21% O_2, 5% CO_2, and 74% N_2 at 60 cycles/min with peak inspiratory pressure of 8 cm H_2O and positive end-expiratory pressure of 3–4 cm H_2O. This resulted in an average effluent perfusate pO_2 = 123.0 ± 3.0, pCO_2 = 21.1 ± 0.5, and pH = 7.41 ± 0.01 (mean ± SEM, n = 10).

Prior to the beginning of each experiment, all lungs were perfused under baseline condition for 30 min and, if necessary, the pH of the perfusate was adjusted to 7.35–7.45 with hydrochloric acid or sodium bicarbonate. Unless specified elsewhere, all drugs were given as bolus injections (0.05–0.20 ml total injection volume) into the pulmonary artery. Hypoxic challenges were performed by switching to a gas mixture containing 0% O_2, 5% CO_2, and 95% N_2 for 5 min, resulting in an average perfusate pO_2 = 41.9 ± 2.4, pCO_2 = 18.6 ± 0.6, and pH = 7.39 ± 0.01 (mean ± SEM, n = 10).

Pulmonary vascular effects of opiate agonists. (1) After the initial 30-min equilibration period, the lungs received 0.05 μg angiotensin II (Human sequence, Sigma Co.) followed by a 4- to 5-min period of alveolar hypoxia. Four lungs then received a 1.5-μg morphine sulfate (Elkins–Sinn, Inc.) injection, followed by an angiotensin II injection, and a hypoxic challenge. Subsequently, 150 μg of morphine was injected followed by two additional pairs of angiotensin II injections and hypoxic challenges. In four other lungs, morphine was injected in doses of 15 and 1500 μg; and finally in four control lungs, two injections of 0.1 ml normal saline were given in place of morphine. In these last eight lungs, the angiotensin II injections and hypoxic challenges were performed in a time course identical to the first four lungs. We compared the perfusion pressures immediately before and 1 min after each injection of morphine, as well as the angiotensin II and hypoxic pressor responses before and after each dose of morphine. At the end of each experiment, the lungs were dissected free and their wet weights were obtained. The lungs were weighed after drying for 3 weeks at room temperature and the wet-to-dry weight ratios were calculated.

(2) Each of eight lungs was exposed to a total of four pairs of alternating angiotensin II (0.05 μg) and hypoxic challenges. At the peak of the second hypoxic vasoconstriction (approximately 2 min after the onset of hypoxia), 1.5 μg and at the peak of the third hypoxic vasoconstriction 150 μg morphine were injected in the first four lungs and similarly 15 and 1500 μg morphine in the other four lungs. The effect of morphine on ongoing hypoxic pulmonary vasoconstriction was assessed by measuring the perfusion pressures 30 sec before and after each morphine injection.

(3) The pulmonary arterial perfusion pressure was increased in four lungs by the addition of up to 1.5 meq potassium chloride to the reservoir. This raised the potassium concentration in the perfusate by approximately 30 m*M*. During the sustained pulmonary vasoconstriction that followed, we added morphine (1.5, 15, 150, and 1500 μg) sequentially to the reservoir to achieve final perfusate concentrations of 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} *M*. The additions of morphine were separated by 3 min and the effect of each dose was assessed by measuring the perfusion pressure initially and 1 min after each morphine addition.

(4) Leucine-enkephalin (acetate salt, Sigma Co.) in doses of 1, 10, and 100 μg was injected into the pulmonary artery in three lungs immediately after the 30-min equilibration period. During subsequent hypoxic challenges in two of these lungs, leucine-enkephalin (1, 10, 100 μg) was injected to test its effect on ongoing hypoxic vasoconstriction.

(5) Dynorphin fragment (Tyr–Gly–Gly–Phe–Leu–DArg–DArg–Ile–DArg–NH_2) was a gift from Dr. Stewart, Department of Pharmacology, University of Colorado Health Sci-

ences Center. In three lungs, the effects of dynorphin were assessed by injections of 1, 10, and 100 μg during the baseline perfusion. The perfusion pressures were recorded initially and 1 min after each injection. In two additional lungs, dynorphin (200 μg) was injected during ongoing hypoxic vasoconstrictions.

Pulmonary vascular effects of naloxone, methylparaben and propylparaben. In a preliminary experiment, we found that Narcan (Endo Pharmaceutical, Inc.), the proprietary preparation of naloxone, caused a marked vasodilation during hypoxic vasoconstriction. Because Narcan contained, in addition to naloxone, 2.0 mg/ml of the preservatives methylparaben and propylparaben in a 9:1 ratio, we obtained methylparaben, propylparaben, and pure naloxone powder from Sigma Company to test each component for vascular effects. Naloxone powder was also obtained from DuPont Pharmaceutical Company, Wilmington, Delaware. Each of these three drugs was dissolved in normal saline to the concentrations contained in the Narcan preparation (naloxone 0.4 mg/ml, methylparaben 1.8 mg/ml, propylparaben 0.2 mg/ml), and injected into the pulmonary artery in a 0.2-ml vol during the peak of a hypoxic pressor response. Perfusion pressures were measured 30 sec before and after each injection.

In four lungs, 80 μg of pure naloxone was injected into the pulmonary artery during baseline perfusion. The perfusion pressure was measured immediately before and 1 min after each injection. In twelve additional lungs, we performed four pairs of alternating angiotensin II (0.05 μg) and hypoxic challenges. During the peak of the second hypoxic vasoconstriction, six lungs received injections of 80 μg of naloxone and the other six lungs injections of 0.2 ml normal saline. The injection of 80 μg of naloxone to the pulmonary artery resulted in a perfusate naloxone concentration of 5×10^{-6} *M*. The first, third, and fourth angiotensin II and hypoxic responses were expressed as percentages of the second response, and compared in the two groups.

Statistical analysis. Data are presented as means ± SE. Student's *t* test and analysis of variance were used to test whether differences between and among groups were significant. Differences were considered significant when $P < 0.05$.

Results. *Pulmonary vascular effects of opiate agonists.* Morphine sulfate (1.5–1500 μg) did not alter the baseline pulmonary perfusion pressure nor did it affect the subsequent angiotensin II and hypoxia induced pulmonary vasoconstrictions. (Results obtained with the 1500-μg dose are shown in Table I). Moreover, the injections of morphine sulfate (1.5–1500 μg) during the hypoxic challenges did not alter the course of ongoing hypoxic pulmonary vasoconstriction (Fig. 1). There was no significant difference between the wet to dry weight ratios of the lungs given morphine or normal saline (5.73 ± 0.27, $n = 8$; vs 6.07 ± 0.50, $n = 4$), indicating that morphine had not caused lung edema.

Additions of potassium chloride to the reservoir raised the pulmonary perfusion pressure by an average of 6.9 ± 0.9 mm Hg ($n = 4$). During the vasoconstriction induced by potassium chloride, additions of 1.5 to 1500 μg morphine sulfate (with resulting concentrations of 10^{-7} to 10^{-4} *M*) caused no significant change in the perfusion pressure (Fig. 2).

In three lungs each, during room air ventilation, the injections 1, 10, and 100 μg of either leucine-enkephalin, a δ-receptor agonist, or dynorphin fragment, a κ-receptor agonist, did not affect the pulmonary artery perfusion

TABLE I. EFFECTS OF MORPHINE SULFATE ON BASELINE PULMONARY ARTERY PERFUSION PRESSURE AND HYPOXIA AND ANGIOTENSIN II-INDUCED VASOCONSTRICTIONS

	n	Before MS[a]	After MS[a] (1500 μg)	
Baseline perfusion pressure	4	5.6 ± 0.3	5.9 ± 0.4	NS[b]
Hypoxic vasoconstriction	4	10.1 ± 0.6	10.4 ± 0.9	NS[b]
Angiotensin II vasoconstriction	4	10.5 ± 0.9	10.0 ± 1.1	NS[b]

[a] Data are presented as means ± SE in mm Hg.
[b] NS = not significant.

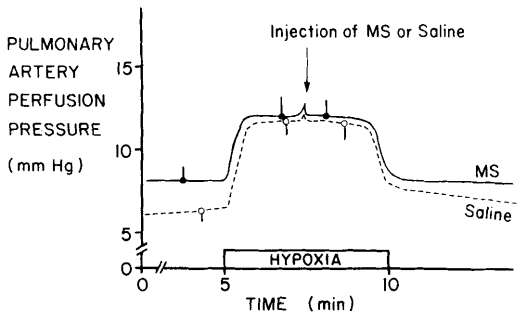

FIG. 1. Effect of morphine sulfate on ongoing hypoxic pulmonary vasoconstriction. Morphine sulfate (MS, 1500 μg, solid line) or saline (dotted line) were injected at the peak of hypoxic vasoconstriction. There was no difference in the perfusion pressures 30 sec before or 30 sec after the injection in the two groups. (n = 4 each group.)

pressures. The perfusion pressures before and 1 min after injections of 1, 10, and 100 μg of leucine-enkephalin were 5.8 ± 0.2, 5.8 ± 0.2, 5.8 ± 0.2, and 5.7 ± 0.2 respectively (mean ± SEM in mm Hg, n = 3). The perfusion pressures before and 1 min after injections of 1, 10, and 100 μg of dynorphin fragment were 6.3 ± 0.9, 6.3 ± 0.9, 6.3 ± 0.9, and 6.7 ± 0.9, respectively (mean ± SEM in mm Hg, n = 3). In two lungs each, injections of leucine-enkephalin (1, 10, and 100 μg) or dynorphin (200 μg) had no effect on ongoing hypoxic vasoconstrictions (data not shown).

Pulmonary vascular effects of naloxone, methylparaben, and propylparaben. Narcan, 0.2 ml, caused vasodilation during hypoxic pulmonary vasoconstriction (Fig. 3a). When each component of Narcan was tested separately during hypoxic vasoconstriction, we

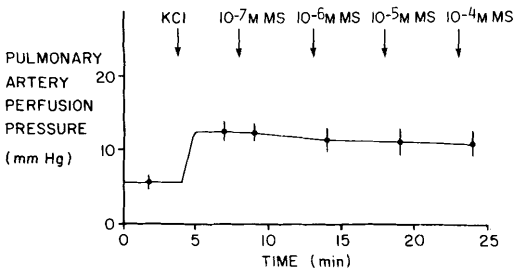

FIG. 2. Effect of morphine sulfate (MS) on potassium chloride (KCl)-induced vasoconstriction. Sequential additions of 10^{-7} to 10^{-4} M MS did not alter the perfusion pressure during KCl-induced vasoconstriction (n = 4).

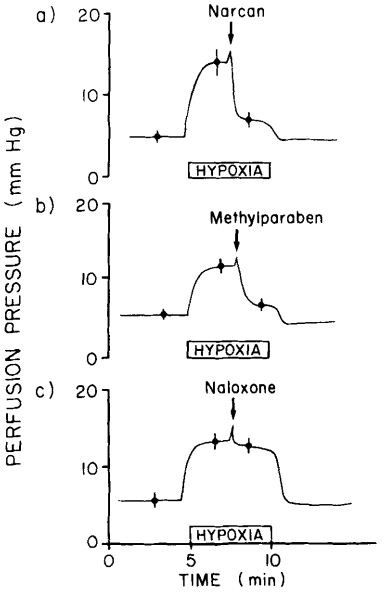

FIG. 3. Effects of Narcan, methylparaben, and naloxone on hypoxic pulmonary vasoconstriction. 0.2 ml of Narcan (a, n = 7) and 0.36 mg of methylparaben (b, n = 5) significantly decreased perfusion pressure when injected at the peak of hypoxic vasoconstriction. 80 μg of pure naloxone (c, n = 6) did not affect ongoing hypoxic pulmonary vasoconstriction.

found that 0.36 mg of methylparaben (the amount contained in 0.2 ml of Narcan) caused vasodilation comparable in degree to 0.2 ml of Narcan (Fig. 3b). Propylparaben had qualitatively similar effects to methylparaben during hypoxic vasoconstriction (results not shown). On the other hand, pure naloxone from two separate sources did not affect ongoing hypoxic vasoconstriction (Fig. 3c). Thus, the vasodilatory property of Narcan during hypoxic vasoconstriction appeared to be entirely accounted for by the preservatives methylparaben and propylparaben.

In four lungs, injections of 80 μg of naloxone did not affect baseline perfusion pressure (results not shown). Comparing the subsequent responses to hypoxia and angiotensin II in lungs given pure naloxone or normal saline, we found that naloxone at 5×10^{-6} M concentration did not significantly alter hypoxia or angiotensin II-induced vasoconstrictions (Fig. 4).

Discussion. Our results showed that acute administrations of morphine sulfate, leucine-enkephalin, and dynorphin were without im-

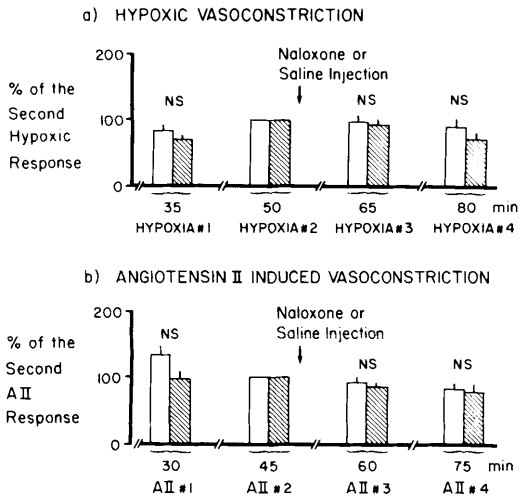

FIG. 4. (a) Sequential hypoxic pulmonary vasoconstriction in lungs treated with naloxone (open bars) or saline (hatched bars). (b) Sequential angiotensin II (0.05 μg)-induced vasoconstriction in the same lungs. All values are expressed as percentages of the second hypoxic or angiotensin II response, and represent mean of six lungs (with standard error). Naloxone or saline were injected during the peak of the second hypoxic response. NS = not significant.

mediate pulmonary vascular effect, and further that morphine did not affect the vasoconstrictions induced by hypoxia, angiotensin II, or potassium chloride. In addition, pure naloxone did not affect the pulmonary perfusion pressure either under baseline condition or during hypoxic vasoconstriction. We conclude that opiate receptor agonists and antagonists do not affect vasoreactivity in the rat pulmonary circulation.

Our attempt to demonstrate opiate effects in the lung circulation was based on the premise that the interactions of exogenous opiate agonists or antagonists with opiate receptors in the lung would result in a detectable change in the pulmonary vascular tone. That no vascular effect was observed could reflect the lack of opiate receptors that function in vasoregulation of the lung circulation or could result from deficiencies in the experimental design including (i) poor sensitivity of the detection system, (ii) use of the inappropriate vascular stimuli, or (iii) poor bioavailability of the exogenously administered drugs. We feel that all of these possibilities are unlikely for the following reasons: (i) Previous studies using the isolated perfused rat lung have shown that the rat lung is extremely sensitive to low doses of vasoactive agents (13). Moreover, the pulmonary vasoconstrictive response to hypoxia is highly susceptible to inhibition by a large variety of agents (12). Yet, despite the relatively high doses of the opiate agonists and antagonist used in our study, we found no effect on either the baseline perfusion pressure or on hypoxic vasoconstriction. (ii) Studies of opiate action in other vascular systems have predominantly noted effects on the baseline vascular tone (3, 5, 10), although reductions of the vasoconstriction induced by potassium-chloride (7) or by nerve stimulation (6) have been reported. Although nerve stimulation is not feasible in the isolated rat lung, we have included studies on potassium chloride-induced vasoconstriction. (iii) All drugs were injected directly into the pulmonary circulation and a wide range of doses were tested for morphine, leucine-enkephalin, and dynorphin. Furthermore, the lungs were perfused with a cell- and plasma-free perfusate to minimize possible binding by plasma proteins or circulating cells. Although the opioid peptides can be potentially inactivated by enzyme systems in the lung (14), the relatively large doses given as a bolus should have overcome this problem. Thus, in our opinion, opiate receptors with significant hemodynamic function are not found in the rat lung circulation.

Our findings differed from those of Gillespie *et al.* who reported that leucine-enkephalin caused vasoconstriction in the isolated rat lungs (15). The reason for this difference is not clear since the experimental protocols were similar and the leucine-enkephalin was obtained from the same source. We also could not corroborate previous reports that naloxone inhibited hypoxic pulmonary vasoconstriction (8). However, in regard to this latter observation, differences in experimental design, species differences, and, possibly, the preparations of naloxone used could account for the disparity in our findings.

Our finding that methylparaben and propylparaben caused vasodilation during hypoxic vasoconstriction is of some interest. Because of their antioxidant property, methylparaben and propylparaben are added as preservatives to a large number of pharmaceutical preparations (9). The amount of the

alkylparabens in these preparations can be quite significant. For example, each milliliter of Narcan contains 0.4 mg of naloxone, 1.8 mg of methylparaben, and 0.2 mg of propylparaben. In our isolated rat lung preparation, the vascular effect of Narcan was entirely attributable to these preservatives.

Other workers have previously noted the effects of these alkylparabens in different experimental preparations. Geddes and Lefcoe (16) reported that the commercial steroid preparation Decadron caused dose-dependent relaxation of isolated guinea pig tracheal chains, and potentiated the relaxation induced by both isoproterenol and dibutyryl cyclic 3',5'-adenosine monophosphate (cAMP). However, further study showed that these effects were due to the methyl- and propylparaben preservatives in Decadron and, in fact, dexamethasone phosphate itself caused contraction of guinea pig trachea. Similarly, Brandt *et al.* (9) and Waters and Harder (10) reported that the alkylparaben in Narcan relaxed isolated human cerebral artery and rat basilar artery *in vitro*. Thus, in a variety of experimental preparations, the alkylparabens are able to cause relaxation of both vascular and nonvascular smooth muscles.

The mechanism for this smooth muscle relaxation effect of the alkylparabens is unclear. Geddes and Lefcoe (16) proposed that alkylparabens may potentiate the effect of isoproterenol and dibutyryl cAMP by inhibition of phosphodiesterase. However, the cAMP content in the tracheal muscles was not measured in their experiments. Brandt *et al.* (9) found that indomethacin did not block the relaxation effect of the alkylparabens. In preliminary experiments in isolated rat lungs, we similarly found that the addition of meclofenamate, another cylooxygenase inhibitor, did not block the vasodilation caused by methylparaben (data not shown). Thus, it would appear unlikely that the vascular action of alkylparabens might be mediated through the production of vasodilating prostaglandins. Whether the vascular effect of the alkylparabens is related to their antioxidant property is unknown.

Although this study found no significant hemodynamic effect in the rat lung circulation for various opiate substances, our finding of the vasodilatory effect of the preservatives methyl- and propylparaben is of potential clinical importance. Recently, based on experimental studies, Faden and others have advocated the use of high-dose naloxone in the treatment of various forms of shock in humans (17). However, only the alkylparaben-containing preparation of naloxone, Narcan, is currently available for clinical use. It is conceivable that, when given in high dose, the vasodilatory action of the alkylparaben preservatives might be detrimental in patients with shock. Alternatively, any beneficial pressor effect of pure naloxone could potentially be attenuated by the concomitant administration of the alkylparabens. Finally, any clinical or experimental studies of vascular action of naloxone should be interpreted with caution if the alkylparaben-containing preparation of naloxone is used.

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