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Morphine potentiates dextromethorphan-induced vasodilation in rat superior mesenteric artery

Saadet Inan, Ronald J. Tallarida*

Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140, USA

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Abstract

The combined action of morphine and dextromethorphan on the superior mesenteric artery was investigated in this study. The artery was cut into rings, placed in a muscle bath and mounted to a force transducer for recording tension. Rings preconstricted with 1 μM phenylephrine produced a dose-dependent relaxation to graded doses of dextromethorphan but showed no response to morphine. An equimolar combination of morphine and dextromethorphan exhibited a marked synergism quantitated by a factor of 3.7 (1.8–7.7, 95% CI). Naloxone, which had no effect on the dextromethorphan dose–response relation, abolished the synergism. Removal of the endothelium produced a slight attenuation of the morphine–dextromethorphan synergism, but the magnitude of this attenuation was the same when dextromethorphan alone was examined in the denuded preparation. In contrast to the marked synergism seen in the mesenteric artery preparation, similar experiments on the carotid artery and the aorta produced only additive interactions.

Keywords: Dextromethorphan; Morphine; Synergism; Mesenteric artery

1. Introduction

Dextromethorphan, the d-isomer of the codeine analog methorphan, is structurally similar to morphine but has low affinity for opioid receptors. It is a widely used over-the-counter cough suppressant that has stimulated renewed interest because of its concomitant use with morphine in treating pain. Dextromethorphan is not addictive and, when used in combination with morphine, blocks morphine tolerance while relieving pain at lower morphine doses (Elliot et al., 1994; Mao et al., 1996). Dextromethorphan is a noncompetitive antagonist of the NMDA receptor. This receptor is one of the receptor subtypes for glutamate, a major excitatory neurotransmitter in the mammalian brain. NMDA receptors are widely distributed throughout the nervous system and are associated with many physiological functions, including pain perception. Because of these properties, there is growing interest in NMDA receptor antagonists. Combinations of NMDA receptor antagonists with opioids and nonsteroidal anti-inflammatory drugs result in marked potentiation of antinociception and clinical analgesia (Price et al., 1996; Grace et al., 1998; Plesan et al., 1998; Henderson et al., 1999; Weinbroom et al., 2001; Weinbroom, 2002; Bulka et al., 2002). Combinations of morphine sulfate and dextromethorphan have been tested on clinical patients for chronic pain with encouraging results (Chevlen, 2000; Katz, 2000).

The interaction between these drugs raises the question of whether or not other end points show this potentiation. Toward that end, our attention was directed to the possible vascular effects of NMDA receptor antagonists and morphine. In contrast to an abundant number of studies of the nervous system effects of the individual and combined actions of these drugs, relatively little has been reported on the vascular effects of morphine and virtually nothing has been published on dextromethorphan's effects on isolated vascular tissues. In that regard, we chose the superior mesenteric artery since that vessel supplies the gastrointestinal tract, a site well known to be affected by morphine. Those studies were followed by additional ones and two other in vitro vascular preparations, the carotid artery and the aorta of the rat. We here report the results when morphine and dextromethorphan, individually and in combination, were administered to these isolated vascular preparations.

* Corresponding author. Tel.: +1-215-707-3243; fax: +1-215-707-7068.
E-mail address: Ronald.Tallarida@temple.edu (R.J. Tallarida).

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2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (mass, 300–350 g) were used in all experiments. The animals were housed two to a gage in a room maintained at temperature 23 °C with a 12-h light–dark cycle. Food and water were available ad libitum.

2.2. Tissue preparation and experimental protocol

The animals were anesthetized and euthanised by exposure to CO_2 (Izzo et al., 2000). Following a medial abdominal incision, the mesenteric bed was reached, allowing removal of the second branch of the superior mesenteric artery. The excised artery was placed in a 4 °C Krebs–Henseleit solution of composition (nM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, D-glucose 10, EDTA 0.038. The arteries were cleaned of any attached connective tissue and cut into rings 2 mm in width. The arterial rings were suspended horizontally between two stainless steel hooks in individual 25-ml organ chambers containing the Krebs–Henseleit solution, which was maintained at 37 °C and oxygenated with a mixture of 95% O_2 + 5% CO_2 in order to achieve a pH of 7.4. The arterial rings were equilibrated for 60 min under zero tension. After this equilibration, the internal circumference was adjusted to $0.90 \times L_{100}$, where L_{100} is the internal circumference in vivo that results from a transmural pressure of 100 mm Hg (Mulvaney and Halpern, 1976). After an additional 30 min, the arterial rings were contracted with 1 μM phenylephrine that produced a maximal tension (approx. 20 mN) within 5 min. The arterial rings were allowed to remain at this tension for an additional 25 min after which test drugs were administered as described in Section 3. Drugs were added in increasing concentrations (cumulative addition) and their maximal relaxation was measured at 5 min after addition (Pratt et al., 1998). In experiments in which naloxone was employed, it was administered 5 min before the test drugs (dextromethorphan, morphine or the combination). In experiments aimed to determine the role of the endothelium, it was removed by gentle rubbing, and then 1 μM acetylcholine was added to the phenylephrine-contracted ring. If the ring did not relax to this addition of acetylcholine that was taken to be evidence that the endothelium was not intact. For the experiments conducted on the mesenteric artery, approximately 200 arterial rings from approximately 60 animals were used. Additional experiments on the carotid artery and the aorta were carried out. These used the same experimental protocol previously described and utilized 43 arterial rings from 21 animals for the carotid artery and 40 rings from 14 animals for the aorta. Data recording was accomplished with PowerLab/8sp and MacLab software (ADInstruments, Grand Junction).

The study reported in this manuscript was carried out to accordance with the Declaration of Helsinki and the Guidance for the Care and Use of Laboratory Animals. All experiments were approved by the Temple University Institutional Animal Care and Use Committee.

2.3. Compounds

Dextromethorphan was purchased from Sigma (St. Louis, MO). Morphine sulfate and naloxone were supplied by NIDA. All drugs were dissolved in distilled water prior to administration.

2.4. Statistical analysis

Dose–response data were analyzed by linear regression of effect on log (dose) from which the potency (D_{50}) was determined along with the S.E.M. In cases where one of the two compounds in the combination exhibited no effect when given alone (e.g., morphine), the assessment of the combination's log (dose)–effect regression was compared to that of the active compound by determining the relative potency as described by Tallarida (2000). All calculations were assisted by employment of the computer package PharmTools Pro (Elkins Park, PA).

3. Results

3.1. Effects of dextromethorphan

Arterial rings of the rat mesenteric artery developed maximal tension, approximately 20 mN, within minutes

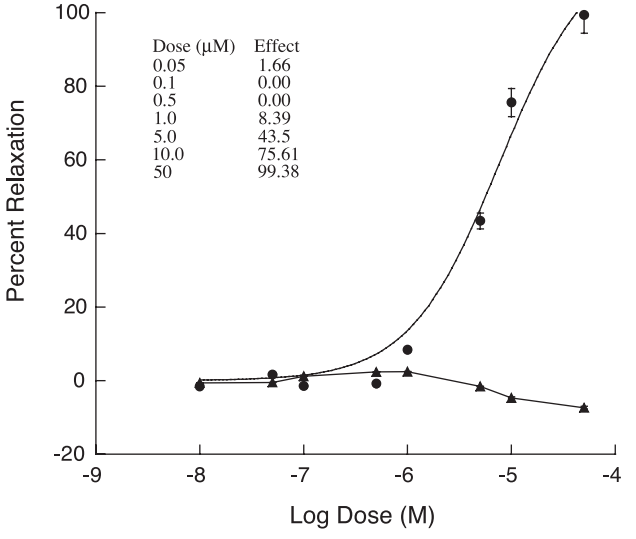

Fig. 1. Percent relaxation (force reduction) of maximally constricted arterial rings in response to graded doses of dextromethorphan (circles) and morphine (diamonds) on isolated superior mesenteric artery. Inset shows the dextromethorphan data set from which the dose for half of maximum effect (D_{50}) = 5.29 ± 0.50 μM (PharmTools Pro).

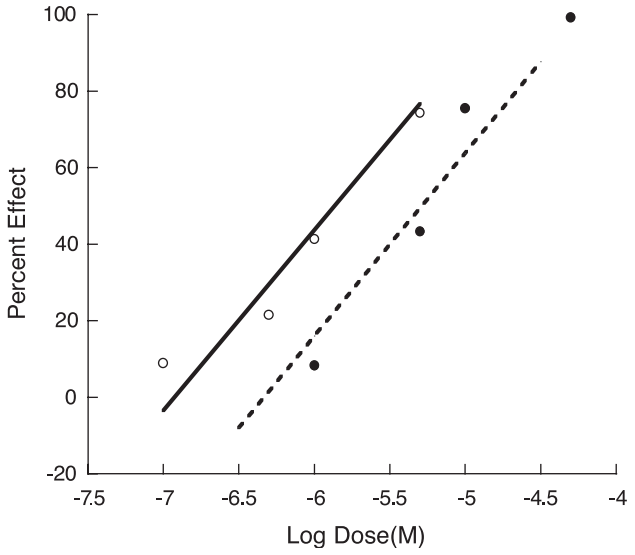

Fig. 2. Linear regressions of effect on log (dose) of dextromethorphan alone (broken line) and together with an equimolar combination of morphine (solid line) on isolated superior mesenteric artery from which the relative potency, indicative of synergism, was calculated.

after dosing with 1 μM phenylephrine. After an additional 25 min at this, maximal tension level dosing with dextromethorphan was begun. Cumulative additions of dextromethorphan produced dose-related relaxation that became maximal at dextromethorphan = 5×10^{-5} M (Fig. 1). The concentration that produced 50% relaxation, denoted D_{50}, was 5.29 + 0.50 μM. In contrast, morphine, in concentrations up to 100 μM, had no relaxing effect on the preconstricted vascular ring as seen in Fig. 1. Hence, the concomitant administration of dextromethorphan and morphine, if simply additive, would be expected to yield a dose–effect relation that is the same as that of dextromethorphan alone. The results of that combination experiment are described below.

3.2. Morphine–dextromethorphan combination

An equimolar combination of dextromethorphan and morphine produced dose-dependent effects that showed enhanced potency as indicated by the leftward shift in the dextromethorphan dose–response curve (Fig. 2). This enhancement was quantitated by determining the relative potency which was calculated to be = 3.7 (1.8–7.7, 95% CI). Because the confidence interval does not include unity, this shift is significant ($P < 0.05$) and indicates synergism for the drug combination.

3.3. Naloxone addition

In order to determine whether the synergism by morphine is a receptor-mediated event, we conducted additional experiments in which naloxone was added to the combination. Naloxone in a concentration of 1 μM completely abolished the combination synergism as noted by the curves of Fig. 3(1) in which it is seen that dextromethorphan, even at the highest concentration tested (10^{-5} M), yielded an effect of only 52% of the maximum. (No larger doses of dextromethorphan were employed in this experimental set since the abolition of the synergism was clearly evident from the curves.) In order to ascertain whether the naloxone effect might be due to an action on dextromethorphan, we conducted experiments involving only dextromethorphan and naloxone, and these results are shown in Fig. 3(2). As seen, two different fixed doses of naloxone, 1 and 3 μM, had no effect on the dextromethorphan dose–response relation.

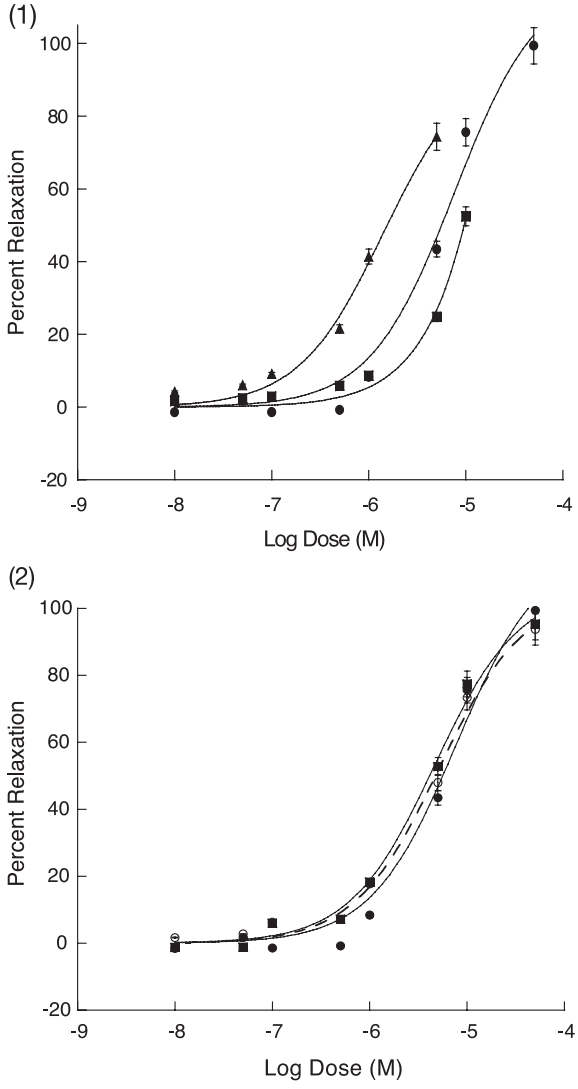

Fig. 3. (1) Graded dose–response curves for dextromethorphan alone (circles), an equimolar combination of dextromethorphan and morphine (triangles), and the combination + 1 μM naloxone (squares) on the isolated superior mesenteric artery. (2) Dose–response curves of dextromethorphan alone (filled circles), dextromethorphan + 1 μM naloxone (open circles) and dextromethorphan + 3 μM naloxone (squares) on rat superior mesenteric artery.

Thus, this morphine antagonist affects the morphine synergism, but not the dextromethorphan-induced relaxation in this arterial segment.

3.4. Role of endothelium

Because the vascular endothelium can release relaxing factors, we conducted a separate set of experiments in which an equimolar combination of dextromethorphan and morphine was tested in normal mesenteric rings and in rings in which the endothelium was removed by gentle rubbing. The denuded preparations exhibited a slight (nonsignificant) shift to the right in the dextromethorphan dose response relation (data not shown). The combination was then examined in both the normal and denuded preparation. The results are shown in Fig. 4 where it is seen that there is a loss of the combination's potency, but the degree of shift is slight and nonsignificant, and is essentially equivalent in magnitude to the shift seen in the dextromethorphan dose–response curve. (Parallel line regression analysis of the two cases did not reach the 95% level of significance.) Thus, there appears to be no obvious role of the endothelium in the morphine–dextromethorphan relaxation synergism in the rat mesenteric artery.

3.5. Aorta and carotid artery

Experiments similar to those described for the mesenteric arterial ring were conducted on rat aorta and carotid arteries. Each of these tissues showed dose-dependent effects (relaxation) to dextromethorphan (Table 1) and no effect for morphine. In contrast to our results in the mesenteric artery, the combination of dextromethorphan and morphine was simply additive in aorta. In the carotid preparation, the mean D_{50} of the dextromethorphan–morphine combination is less than that of dextromethorphan alone, suggestive of a synergistic interaction, but the difference was not significant.

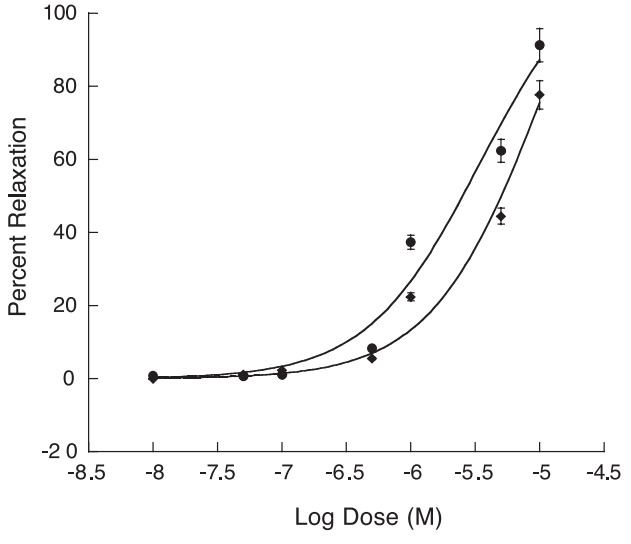

Fig. 4. Graded dose–response curves of an equimolar combination of dextromethorphan and morphine in endothelium intact rings of the rat mesenteric artery (circles) and in rings in which the endothelium was removed (diamonds).

Table 1
Dose–response (relaxation) data for dextromethorphan in preconstricted isolated rat vessels

	Dose (μM)	Percent relaxation
Carotid artery[a]	5	5.55
	10	40.6
	50	64.0
	$D_{50} = 23.9 \pm 9.44$	
Aorta[b]	5	26.7
	10	52.2
	50	82.5
	$D_{50} = 11.5 \pm 2.00$	

[a] The carotid combination (equimolar) gave $D_{50} = 11.7 \pm 7.7$ μM, a lower mean, but not significantly different from that of dextromethorphan alone.

[b] In aorta, the combination gave $D_{50} = 8.40 \pm 5.4$ μM, which was not significantly different from that of dextromethorphan alone.

4. Discussion

Dextromethorphan produced a dose-dependent relaxation in all isolated vessel rings tested whereas morphine had no effect in any of these preparations. The results in the mesenteric rings were unusual in that the addition of morphine produced an enhanced response to dextromethorphan. Because morphine was found to have no effect on this tissue (or on the other tissues tested), a simply additive interaction is one in which the dextromethorphan dose–response relation is the same as that of dextromethorphan alone. This was not the case in the mesenteric artery; thus, there is synergism between morphine and dextromethorphan in this vessel's relaxation response. Further, this synergism depends on activation of a morphine receptor since its block by naloxone completely abolished the synergism. This result suggests that morphine–receptor binding takes place in the artery even though this binding has no overt effect on vascular contractility. It is not known whether the synergism seen in the vessel has the same mechanism as that seen in the combination's analgesic action. In that regard, we examined the possible role of nitric oxide (NO) by removing its endothelial source. Our findings with endothelium-deficient preparations did not reveal an obvious role of the endothelium in this synergistic interaction; i.e., the diminution of the potency enhancement of the combination when the endothelium was removed was also seen in responses to dextromethorphan alone. In contrast to the synergism exhibited by this combination in the superior mesenteric artery, similar combination experiments in isolated carotid and aortic rings failed to show a difference from simple additivity.

The mechanism of the dextromethorphan effect on blood vessels is not known. That action might involve the NMDA

receptor, but we did not specifically test for that. However, this demonstration of potentiation in vascular dilation, when viewed against this same combination's analgesic potentiation, causes us to speculate that both effects may involve the NMDA receptor in some way. Further study is needed to test this speculation. While very little is known about the actions of opioids on blood vessels (and virtually nothing has been published on dextromethorphan's vascular effects), there is evidence that blood vessels express opioid receptors and that these are somehow related to the release of NO. That work with human (and rat) endothelial cell cultures demonstrates that morphine exerts modulatory control over endothelial cells that induce NO production (Stefano et al., 1995, 1998). However, the synergism of the combination in the mesenteric artery does not appear to be due to NO. Synergism is the result whenever the concomitant presence of two chemicals produces effects that exceed what is expected from their individual potencies. In this case, it is especially evident since one of the two agents (morphine) had no potency and, thus, the additive combination's dose–effect relation is the same as that of dextromethorphan. The potency enhancement produced by morphine in the mesenteric tissue, which is clearly indicated from data with low scatter, provides evidence of synergism between morphine and dextromethorphan that requires activation of the morphine receptor in the rat blood vessel that supplies the gastrointestinal system.

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