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Endomorphins inhibit contractile responses of rat thoracic aorta rings induced by phenylephrine and angiotensin Ⅱ *in vitro*[1]

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KEY WORDS opioid peptides; endomorphins; morphine; phenylephrine; angiotensin Ⅱ; thoracic arteries; nitric oxide

ABSTRACT

AIM: To study the effects of opioid receptor agonists endomorphin-1 and -2 on contractile responses of rat thoracic aorta rings to phenylephrine (PE) and angiotensin Ⅱ (Ang Ⅱ), and their possible mechanism *in vitro*. **METHODS**: Isometric tension recording was progressed in thoracic aorta rings from Wistar rats. **RESULTS**: Pretreatment of morphine, endomorphin-1 and -2 (0.1, 1, and 10 μmol/L) could inhibit the contractile responses of the endothelium-intact aorta rings to PE (0.1 μmol/L) and Ang Ⅱ (1 μmol/L) in a concentration-dependent manner ($P < 0.01$), but could not inhibit the contraction of rings without endothelium ($P > 0.05$). Naloxone (1 μmol/L) could partially antagonize the effects of endomorphine-1 and -2 ($P < 0.01$). N^{ω}-nitro-*L*-arginine (*L*-NNA, 10 μmol/L) or endothelial rubbing could completely blocked the effects of morphine, endomorphine-1 and -2 ($P < 0.01$). **CONCLUSION**: Endomorphin-1 and -2 could inhibit PE- and Ang Ⅱ-induced contractions of rat aorta rings, which was partially by naloxone-sensitive mechanism and related to the release of nitric oxide from vascular endothelium.

INTRODUCTION

Endogenous opioid system has been shown to play a role in the regulations of vascular smooth muscle tone, regional blood flow, and blood pressure in normal and hypertension states[1-4]. Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2; EM-1) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2; EM-2) are novel endogenous opioid peptides. They differ from conventional endogenous opioid receptor ligands in their *N*-terminal sequence (Tyr-Pro *vs* Tyr-Gly), tetrapeptide length, and *C*-terminal amidation. Based on the potent and selective activity of the peptides on the μ-opioid receptor[5], it has been suggested that the discovery of endomorphin-1 and endomorphin-2 lead to development of new analgesics with less addictive properties than traditional opioid agonists. To achieve this goal, it is necessary to understand the physiological and pharmacological functions of EM-1 and EM-2 in cardiovascular system. It has been reported that endomorphins can decrease systemic arterial pressure in rabbit[6], rat[7], cat[8], and mouse[9], and have significant vasodilator activity in the hindquarters vascular bed of the rat[10]. However, the mechanisms of opioid peptides to mediate vasodepressor responses are not well understood.

To investigate the role of endomorphins in modulating the vascular tone and their possible mechanism *in vitro*, the effects of EM-1 and EM-2 on the vascular tone were determined in aorta rings of rats with or without endothelium, and compared with morphine.

MATERIALS AND METHODS

Drugs and chemicals EM-1, EM-2, and angiotensin Ⅱ were synthesized by solid-phase method, then purified and determined by HPLC in our laboratory (>95 %), their structures were verified by mass spectrometry and amino acid analysis. Morphine hydrochloride (Mor) and phenylephrine hydrochloride were purchased from Shenyang First Pharmaceutical Factory. Naloxone hydrochloride (Nx) and N^{ω}-nitro-*L*-arginine

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(*L*-NNA) were purchased from Sigma Chemical Co.

Animals Male Wistar rats (220 – 280 g) were purchased from Medical Laboratory Animal Center of Lanzhou Medical College (Grade Ⅱ, Certificate No 14-005). Before use, rats were housed in individual cage and maintained on 12-h/12-h light/dark schedule, with food and water provided *ad libitum*.

Vascular reactivity experiments Thoracic aortae of rat were free from surrounding tissues and cut into cylindrical segments 4 – 5 mm long. The endothelium was removed by rubbing with cottoo swabs in the rings denuded of endothelium. The segments were mounted on 10 mL bath containing Krebs' solution with following composition (mmol/L): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 25.0, and glucose 11.0, at (37.0 ± 0.5) ℃, and gassed continuously with a mixture of 95 % O_2 and 5 % CO_2 (pH 7.30 – 7.40). A resting tension of (1.0 ± 0.3) g was applied to the vascular segments. The rings were equilibrated for 90 min and the output from the force transducer was observed and recorded in a computer with MS302 system (Guangdong Pharmaceutical College). Before each experiment, contractile responses to KCl 30 mmol/L were used to assess vascular smooth muscle function, whereas relaxation responses to acetylcholine (1 μmol/L) in rings precontracted with PE (0.1 μmol/L) were used to test endothelial integrity. Following these, different concentrations of EM-1, EM-2, and morphine (0.1, 1, and 10 μmol/L) were added at 5 min before the rings were contracted by PE (0.001 – 10 μmol/L) and Ang Ⅱ (1 μmol/L). In other test groups, Nx (1 μmol/L) or *L*-NNA (10 μmol/L) was added to the bath 5 min prior to morphine, EM-1, and EM-2.

Data analysis All responses were expressed as a percent decrease of the tension induced by phenylephrine or Ang Ⅱ. Results were expressed as $x \pm s$. Intergroup differences were analyzed using one-way analysis of variance and followed by paired *t*-test.

RESULTS

Effects of EM-1 and EM-2 on contractile responses of rat aorta rings to PE In endothelium-intact aortic rings, EM-1, EM-2, and morphine at concentrations of 0.1, 1, and 10 μmol/L could inhibit the contractile responses of rings to PE and the effects were concentration-dependent. The results were summarized in Fig 1. In PE (0.1 μmol/L) groups, the IC_{50} of morphine, EM-1, and EM-2 was 13.9, 0.92, and 1.74 μmol/L, respectively.

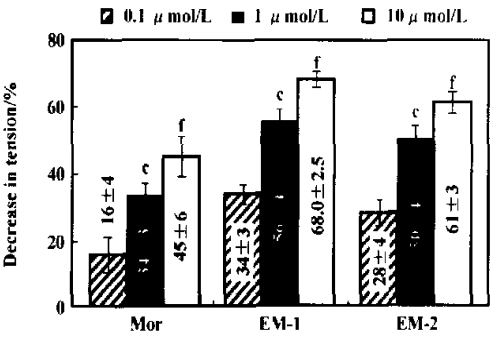

Fig 1. Concentration-dependent responses of morphine, EM-1, and EM-2 to isolated aorta rings contracted by PE (0.1 μmol/L). $n = 10$. $\bar{x} \pm s$. Vascular tension changes to morphine and opioid peptides are expressed as percent decrease rate, the maximal response of aortic rings to PE was taken as 100 %. $^cP < 0.01$ *vs* 0.1 μmol/L group. $^fP < 0.01$ *vs* 1 μmol/L group.

Naloxone (1 μmol/L) can partially antagonize the inhibitory effects of morphine ($P < 0.01$), EM-2 ($P < 0.01$), and EM-1 ($P < 0.05$) (Tab 1). Administration of *L*-NNA (10 μmol/L) or endothelial rubbing was associated with a rapid, robust, and reproducible vasoconstrictor response in all aortic rings examined. In contrast to no *L*-NNA-pretreated and endothelium-intact aortic rings, administration of *L*-NNA or endothelial rubbing abolished the inhibitory effects of morphine, EM-2, and EM-1 on the tension of the rings contracted by PE (0.001 – 10 μmol/L).

Effects of morphine, EM-1, and EM-2 on contractile responses to Ang Ⅱ In control group, Ang Ⅱ (1 μmol/L) increased tension of rings to maximal values of (1539 ± 209) mg. In test rings, increasing morphine, EM-1, and EM-2 from 0.001 to 1 μmol/L reduced tension of rings in a concentration-dependent manner ($P < 0.01$). However, the response to morphine was minimal, the tension reduced by 140 – 513 mg compared with normal saline group, whereas EM-1 and EM-2 reduced by 220 – 538 mg and 404 – 556 mg of tension respectively. Their IC_{50} were 0.29, 0.08, and 0.005 μmol/L, respectively. At lower concentrations (0.001 to 0.1 μmol/L), the effect of EM-2 was the strongest, secondly EM-1, morphine ($P < 0.01$), but there were no significant differences at 1 μmol/L between EM-2 [(69 ± 9) %], EM-1 [(69 ±

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Tab 1. Influence of naloxone (1 μmol/L) and *L*-NNA (10 μmol/L) on the inhibitory roles of morphine, EM-1, and EM-2 (at 1 μmol/L) in the isolated rat aortic rings contracted by PE (0.001 – 10 μmol/L). ETR is endothelial-rubbing groups. Vascular tension responses to morphine and opioid peptides are expressed as percent decrease rate. $n = 10$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

	Concentrations of PE/μmol·L^{-1}				
	0.001	0.01	0.1	1	10
Mor	82 ± 7	53 ± 5	34 ± 3	28 ± 1.8	13.0 ± 1.4
Nx + Mor	63 ± 4^c	49.0 ± 2.5^c	15 ± 4^c	8.0 ± 2.4^c	2.7 ± 1.5^c
L-NNA + Mor	27 ± 3^c	24 ± 6^c	−6.3 ± 1.4^c	−37 ± 2.3^c	−45 ± 3^c
ETR	5.0 ± 2.3^c	−7 ± 4^c	−22 ± 6^c	−49 ± 7^c	14.0 ± 1.3^c
EM-1	100 ± 11	93 ± 6	56 ± 4	43 ± 6	28 ± 7
Nx + EM-1	77 ± 5^c	62 ± 11^c	44 ± 6^c	37 ± 4^c	21 ± 3^c
L-NNA + EM-1	−8.0 ± 1.2^c	−13 ± 5^c	−51 ± 3^c	−40 ± 2^c	−36.0 ± 2.5^c
ETR	−13.0 ± 2.6^c	−79 ± 4^c	−85 ± 5^c	−97 ± 20^c	−10 ± 10^c
EM-2	97 ± 6	57 ± 4	50 ± 4	38 ± 8	27 ± 3
Nx + EM-2	67 ± 5^c	29 ± 4^c	18 ± 8^c	11.0 ± 1.9^c	5.0 ± 1.2^c
L-NNA + EM-2	9.0 ± 2.0^c	−0.8 ± 1.4^c	−37 ± 7^c	−54 ± 5^c	−52 ± 6^c
ETR	−33 ± 9^c	−26 ± 5^c	−11.0 ± 2.5^c	−25 ± 6^c	9 ± 4^c

6) %], and morphine [(60 ± 4) %] ($P > 0.05$, Fig 2).

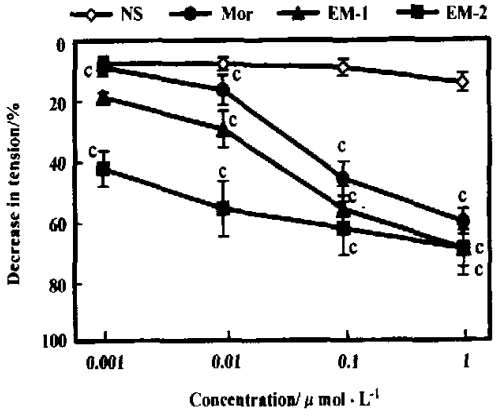

Fig 2. Concentration-response curve for morphine, EM-1, and EM-2 to isolated aorta rings contracted by Ang Ⅱ (1 μmol/L). Vascular tension changes to morphine and opioid peptides are expressed as percent decrease rate. The maximal response of aorta rings to Ang Ⅱ was taken as 100 %. NS is normal saline group. $n = 10$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* NS.

In the presence of naloxone or *L*-NNA and endothelial rubbing, the roles of EM-1, EM-2, and morphine in contractile responses to Ang Ⅱ were also investigated (Fig 3). In contrast to the control, the administration of naloxone (1 μmol/L) did not alter the vasodepressor response to morphine ($P > 0.05$), but had

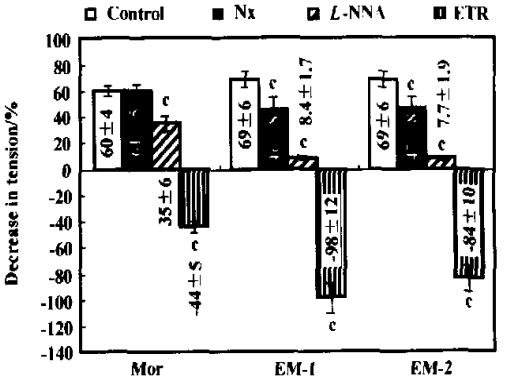

Fig 3. Influences of naloxone (1 μmol/L), *L*-NNA (10 μmol/L), and endothelial rubbing (ETR) on vasodepressor responses to morphine, EM-1, and EM-2 (at 1 μmol/L) in aorta rings contracted by Ang Ⅱ. $n = 10$. $\bar{x} \pm s$. Responses were compared before and after administration of naloxone and *L*-NNA and between the rings with and without endothelium. $^cP < 0.01$ *vs* control.

significant effect on the responses to EM-1 and EM-2 ($P < 0.01$). In the presence of naloxone, the inhibitory rates of EM-1 and EM-2 decreased from (69 ± 6) % to (46 ± 8) % and from (69 ± 6) % to (47 ± 8) %, respectively. In the presence of *L*-NNA (10 μmol/L), the tensions of aorta rings contracted by Ang Ⅱ were remarkably increased. The inhibitory rates of morphine, EM-1, and EM-2 were only (35 ± 6) %, (8.4 ± 1.7) %, and (7.7 ± 1.9) %, respectively. Compared

with no administration of *L*-NNA, there was significant difference ($P < 0.01$). In aorta rings denuded of endothelium, the vasoconstrictor response to Ang Ⅱ was remarkably potentiated, and the inhibitory rates of morphine, EM-1, and EM-2 were abolished (-44 ± 5) %, (-98 ± 12) % and (-84 ± 10) %.

DISCUSSION

The result of the present study showed that endogenous selective μ-opioid receptor agonists endomorphins 1 and 2 inhibited contractile responses to PE and Ang Ⅱ in endothelium-intact aortic rings from rats. In addition, we first observed the blocking action of the nitric oxide synthase inhibitor *L*-NNA on endomorphins, and the interaction between the endomorphins and Ang Ⅱ in isolated aortic rings.

In the presence of morphine, endomorphin-1, and -2, the contractile responses of the rat aorta rings *in vitro* to PE were decreased in a concentration-dependent manner (Fig 1), both endomorphin-1 and -2 (inhibitory rates were 34 % – 68 % and 28 % – 61 %) were more potent than morphine (16 % – 45 %) in PE-contracted aorta rings ($P < 0.01$), there was no significant difference between endomorphins ($P > 0.05$). These results were in accordance with those reports in the hindquarters vascular bed and the systemic arterial pressure of the rat[7,10], exhibited that morphine, endomorphin-1, and -2 had vasodepressor activity not only *in vivo*, but also *in vitro*. Morphine is nonselective ligand for the μ-, κ-, and δ-receptors, its selectivity for μ-opioid receptor is less potent than EM-1 and -2[5], these results suggest that the vasodepressor activity of opioid be related to μ-opioid receptor.

Morphine, EM-1, and EM-2 could also attenuate the vasoconstrictor effect of Ang Ⅱ in a concentration-dependent manner. EM-1 and EM-2 were more potent than morphine in decreasing the tensions of the rings, especially at low-concentration (0.001 – 0.1 μmol/L, $P < 0.01$), the potency rank order was EM-2 > EM-1 > morphine, but their inhibitory effects were almost equal at 1 μmol/L. The results suggested that the inhibitory effects of opioid agonists on the rings be close to maximum when opioid receptors were nearly saturated at higher concentration. In contrast to control, the effects of EM-1 and EM-2 on contractile responses to PE or Ang Ⅱ were partially antagonized by naloxone ($P < 0.01$) (Tab 1, Fig 3). These results suggested that morphine, endomorphin-1, and -2 decrease the tensions of isolated rat aortic rings at least in part by a naloxone-sensitive mechanism. Although μ_1 and μ_2-opioid peptide receptors in endothelium were not detected in normal condition[11,12], it has been reported that vasorelaxant responses to endomorphins in isolated rat aorta were endothelium-dependent[13]. However, naloxone could not alter the response to morphine in Ang Ⅱ-contracted rings ($P > 0.05$). The relations among morphine, naloxone, and Ang Ⅱ still need to be further confirmed.

Nitric oxide (NO) is one of the most important regulators of vascular tone. The vascular endothelium synthesizes NO from *L*-arginine via the enzyme nitric oxide synthase (NOS), and NO modulates vascular tone as endothelium-derived relaxing factor (EDRF), and nitric oxide synthase inhibitor *L*-NNA suppresses production of NO by inhibiting the activity of NOS. As shown in Tab 1 and Fig 3, the pretreatment with *L*-NNA or endothelial rubbing abolished vasodepressor responses to EM-1 and EM-2 in isolated aortic rings from rats and potentiated the contraction induced by PE and Ang Ⅱ. It has been reported that vasorelaxant responses to endomorphins in isolated rat aorta were endothelium-dependent and similar to those induced by acetylcholine[13]. Our results further confirmed this. Since the rat aorta rings were not controlled by nervous system *in vitro*, the present results implicated that the activities of morphine, EM-1, and EM-2 had a close relation with release of NO from endothelium.

In conclusion, this study reported that opioid ligands EM-1 and EM-2 suppressed PE- and Ang Ⅱ-induced contraction of rat aorta rings in a concentration-dependent manner. The activities of them were naloxone-sensitive and were markedly attenuated or abolished in aorta rings pretreated with *L*-NNA or endothelial rubbing. The results of this study indicate that EM-1 and EM-2 may play an important role in modulating the vascular tone by the release of NO from endothelium.

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内吗啡肽抑制苯肾上腺素和血管紧张素Ⅱ诱导的离体大鼠胸主动脉环收缩[1]

R96 A

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关键词　阿片样肽类；内吗啡肽类；吗啡；苯肾上腺素；血管紧张素Ⅱ；胸部动脉；一氧化氮

目的：研究吗啡、内吗啡肽-1和内吗啡肽-2对由苯肾上腺素(PE)和血管紧张素(Ang)Ⅱ诱导离体大鼠胸主动脉环收缩的抑制作用．**方法**：离体血管环张力试验．**结果**：与对照组相比，吗啡、内吗啡肽-1和内吗啡肽-2的预处理（0.1 – 10 μmol/L）能明显降低由PE(0.1 μmol/L)和Ang Ⅱ（1 μmol/L）（$P < 0.01$）诱导离体大鼠胸主动脉环的张力，但是不能减少去内皮血管的张力．纳络酮(1 μmol/L)能部分阻断内吗啡肽-1和内吗啡肽-2的抑制作用（$P < 0.01$），N^{ω}-nitro-*L*-arginine (10 μmol/L)或血管环去内皮能完全阻断这种作用（$P < 0.01$）．**结论**：内吗啡肽-1和内吗啡肽-2通过纳络酮敏感方式抑制由PE和Ang Ⅱ诱导的离体大鼠胸主动脉环收缩，这种抑制作用可能与血管内皮NO的释放有关．

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