侧脑室注射内吗啡肽-1 对麻醉大鼠血压的影响

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摘要: 目的 观察侧脑室注射内吗啡肽-1(EM-1)对麻醉大鼠血压的影响,并初步探讨其作用机理。方法 侧脑室埋植导管给药,颈动脉插管测血压。结果 icv EM-1 剂量依赖、纳洛酮敏感地降低麻醉大鼠的血压。icv 或 iv 酚妥拉明、普萘洛尔和 iv *L*-NNA 对 EM-1 引起的血压降低反应无影响;给予阿托品(icv 25 μg·kg^{-1};或 iv 50 μg·kg^{-1})和切断双侧迷走神经减弱 EM-1 引起的血压降低反应。结论 icv EM-1 可引起麻醉大鼠血压降低;此效应由阿片受体介导,有中枢 M 受体的参与,通过兴奋迷走神经所致。

关键词: 内吗啡肽-1; 降血压作用; 阿片受体; 中枢 M 受体

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静脉或中枢注射阿片类物质,如 DAMGO([*D*-Ala^2, *N*-Me-Phe^4, Gly-ol^5]enkephalin), DPDPE([*D*-Pen^2, *D*-Pen^5]enkephalin), U50488 H,强啡肽和孤啡肽等,可通过 μ, δ, κ 和 ORL1 等阿片受体引起血压升高或降低;并且由于动物种属、剂量、给药途径、注射位点、麻醉或清醒状态的差异,可通过不同的机制而引起不同的效应,例如对清醒大鼠,icv 强啡肽 A_{1-13} 引起血压升高,而 iv 则引起血压降低[1];iv 孤啡肽对清醒大鼠降血压[2],对清醒绵羊升血压[3],而孤束核内注射孤啡肽对麻醉大鼠升血压[4]。内吗啡肽-1(endomorphin-1, EM-1, Tyr-Pro-Trp-PheNH_2)是最近发现的 μ 阿片受体内源性配体[5],我们以前的工作[6]表明,iv EM-1 主要通过促进血管内皮细胞释放 NO 显著降低麻醉大鼠的血压,但 EM-1 是否有中枢性降压作用未见报道。本文观察了中枢注射 EM-1 对麻醉大鼠血压的影响,初步探讨其作用机制,并比较与 iv EM-1 引起降血压作用的异同。

材 料 和 方 法

药品与仪器 内吗啡肽-1(endomorphin-1, EM-1),由本实验室合成,HPLC 分析纯度 > 95 %;硫酸阿托品(atropine sulfate, Atro):湖南洞庭药业有限公司;酚妥拉明(phentolamine, Phen):Ciba 公司;盐酸普萘洛尔(propranolol hydrochloride, Prop)、N^{ω}-硝基-*L*-精氨酸(N^{ω}-nitro-*L*-arginine, *L*-NNA)和盐酸纳洛酮(naloxone hydrochloride, Nx)均为 Sigma 公司产品。所有药品均用生理盐水(NS)配制。江湾 I 型 C 立体定位仪,江湾仪器厂;YP-1 型压力传感器和 LMS-2B 型二道生理仪,成都仪器厂。

动物 体重 200 - 250 g 的 ♂ Wistar 大鼠,(Certificate No.) 14 - 005 由兰州医学院提供。

侧脑室埋植导管[7] 大鼠用 20 %氨基甲酸乙酯(1.0 - 1.2 g·kg^{-1}, ip)麻醉,俯卧位固定在江湾 I 型 C 立体定位仪上,暴露颅骨,将内径 0.5 mm、外径 0.8 mm 的不锈钢导管插入侧脑室(坐标:P 1.0 mm, R 1.5 mm, H 4.0 mm, Paxinos-Watson 图谱),用 502 胶混合牙托粉固定在颅骨上,以备 icv 给药用。术后恢复 3 天进行实验。

平均动脉压实验[8] 恢复良好的大鼠,用 20 %氨基甲酸乙酯(1.0 - 1.2 g·kg^{-1}, ip)麻醉,作气管插管,分离左侧颈总动脉,插入充满 1 000 u·L^{-1} 肝素钠生理盐水的导管,经 YP-1 型压力换能器与 LMS-2 型二道生理仪相连,记录收缩压、舒张压及心率,平均动脉压 = 舒张压 + (收缩压 - 舒张压)/3。待血压稳定后进行实验,整个实验过程肛温稳定在(37.5 ± 0.5) ℃。部分动物还作了右侧颈外静脉插管或切断双侧迷走神经。icv 体积 10 μL, 1 min 内注毕;iv 体积 100 μL, 15 s 内注毕。

实验结束后经侧脑室导管注入 5 %溴酚蓝 10

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μL,以确定药液是否进入侧脑室。

统计方法　实验数据以 $\bar{x} \pm s$ 表示,用配对 t 检验比较组内实验前后差异,用方差分析和两样本均数 t 检验比较组间差异。

结　果

1　icv EM-1 后血压的变化

icv EM-1 0.5 μg·kg^{-1} 对麻醉大鼠的舒张压、收缩压、平均动脉压及心率无影响($P > 0.05$) ;icv 5 ,20 和 50 μg·kg^{-1} 引起舒张压、收缩压、平均动脉压及心率均降低,脉压差增大($P < 0.05$) ;平均动脉压变化值分别为(- 12.4 ± 1.1) mmHg,(- 21.3 ± 3.0) mmHg (1 mmHg = 0.1333 kPa) 和(- 32.6 ± 2.0) mmHg,此效应可被 Nx(250 μg·kg^{-1} ,icv) 阻断(图 1) 。icv EM-1 20 μg·kg^{-1} 后 30 - 60 s 平均动脉压开始下降,4 - 6 min 达到最低值,16 - 20 min 后完全恢复(图 2) ;舒张压、收缩压及心率的变化分别为(- 22.9 ± 2.3) mmHg,(- 19.4 ± 3.2) mmHg 和(84 ± 11) beat·min^{-1} (表 1) ,脉压差由给药前的(32.3 ± 5.1) mmHg 增大至(35.6 ± 3.3) mmHg。

Table 1　Effects of icv endomorphin-1 (EM-1) 20 μg·kg^{-1} on systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in anesthetized rats

Group	SBP/mmHg	DBP/mmHg	SBP-DBP/mmHg	MAP/mmHg	HR/beat·min^{-1}
Control					
Before treatment	114 ± 3	82 ± 4	31 ± 3	93 ± 3	372 ± 22
After treatment	112 ± 5*	83 ± 4*	30.4 ± 2.2*	92 ± 4*	374 ± 30*
EM-1					
Before treatment	114 ± 4	83 ± 7	32 ± 5$^{\#}$	93 ± 5	379 ± 22
After treatment	95 ± 4***	61 ± 6***	36 ± 3$^{**\#\#\#}$	71 ± 4***	296 ± 27***

$\bar{x} \pm s$, $n = 7$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ *vs* before treatment; $^{\#}$ $P > 0.05$, $^{\#\#\#}$ $P < 0.01$ *vs* control.
SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate

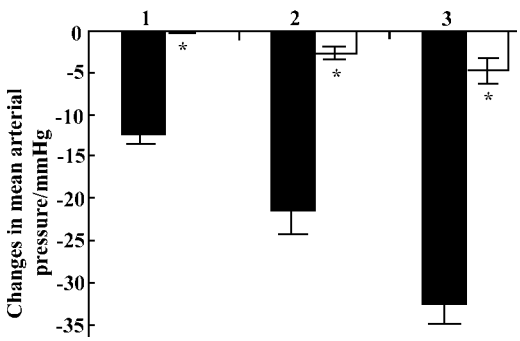

Figure 1　Effects of icv endomorphin-1 (EM-1) on mean arterial pressure in anesthetized rats
$\bar{x} \pm s$, $n = 7$. * $P < 0.01$ *vs* EM-1. EM-1: (1) 5 μg·kg^{-1}; (2) 20 μg·kg^{-1}; (3) 50 μg·kg^{-1}. ■ EM-1; □ Naloxone + EM-1. Naloxone was injected at a dose of 250 μg·kg^{-1} (icv). After 10 min, EM-1 was injected (icv). The decrease in mean arterial pressure induced by icv EM-1 was blocked by naloxone

2　icv Nx, Atro, Phen, Prop 对 icv EM-1 引起的血压降低的影响

icv Nx(250 μg·kg^{-1}) , Atro(25 μg·kg^{-1}) , Phen (50 μg·kg^{-1}) 和 Prop(50 μg·kg^{-1}) 作为预处理,平均动脉压变化最大值分别为(1.2 ± 0.7) mmHg, (- 2.93 ± 0.8) mmHg, (- 4.5 ± 0.9) mmHg 和 (- 5.9 ± 1.1) mmHg,10 - 15 min 后平均动脉压恢复至基础值($P > 0.05$,与预处理前比较) ,此时再 icv EM-1 20 μg·kg^{-1}。结果表明 Phen 和 Prop 对 EM-1 引起的血压降低无影响,Nx 和 Atro 减弱 EM-1 引起的血压降低(表 2) 。

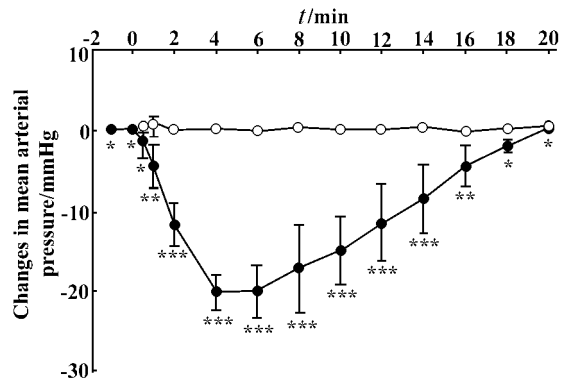

Figure 2　Time-response curve of the decrease in mean arterial pressure of icv endomorphin-1 (EM-1) 20 μg·kg^{-1} in anesthetized rats
$\bar{x} \pm s$, $n = 7$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ *vs* NS. •—• EM-1; ○—○ NS. After icv endomorphin-1 at the dose of 20 μg·kg^{-1}, the mean arterial pressure began to decrease within 30 - 60 s and reached the lowest level within 4 - 6 min. Then the mean arterial pressure returned slowly to baseline value over a 16 - 20 min period

Table 2 Effects of icv naloxone, atropine, phentolamine and propranolol on the depressor action induced by endomorphin-1 (EM-1) (icv, 20 μg·kg^{-1}) in anesthetized rats

Treatment	n	Mean arterial pressure / kPa		
		Baseline value before pretreatment	Baseline value after pretreatment	Maximal change of endomorphin-1
EM-1 after NS	6	99.3 ±7.0	99.7 ±8.5 #	- 20.8 ±1.8
EM-1 after Nx	7	97.6 ±7.4	97.3 ±8.0 #	- 3.2 ±0.8 *
EM-1 after Atro	7	93.7 ±7.1	94.4 ±7.4 #	- 3.5 ±1.7 * *
EM-1 after Phen	7	97.7 ±3.0	98.6 ±3.2 #	- 18.9 ±0.9 *
EM-1 after Prop	7	94.5 ±6.7	94.1 ±8.4 #	- 20.3 ±3.0 *

$\bar{x}$ ± s. # $P > 0.05$ vs baseline value before pretreatment; * $P > 0.05$, * * $P < 0.01$ vs EM-1 after NS. NS, naloxone (Nx), atropine (Atro), phentolamine (Phen) or propranolol (Prop) was icv injected and the mean arterial pressure recovered to the baseline value before pretreatment over a 10 - 15 min period ($P > 0.05$). When the mean arterial pressure recovered, endomorphin-1 (20 μg·kg^{-1}, icv) was injected. Nx: 250 μg·kg^{-1}; Atro: 25 μg·kg^{-1}; Phen: 50 μg·kg^{-1}; Prop: 50 μg·kg^{-1}

3 iv Atro, Phen, Prop, *L*-NNA 和切断双侧迷走神经对 icv EM-1 引起的血压降低的影响

iv Atro(50 μg·kg^{-1}), Phen(100 μg·kg^{-1}), Prop(200 μg·kg^{-1}) 和 *L*-NNA(25 mg·kg^{-1}) 作为预处理,预处理后平均动脉压变化最大值分别为(- 3.2 ± 1.2) mmHg,(- 4.6 ±2.1) mmHg,(- 3.8 ±0.9) mmHg 和(1.8 ±0.5) mmHg,10 - 15 min 后平均动脉压恢复至基础值($P > 0.05$,与预处理前比较)。切断双侧迷走神经组基础平均动脉压为(97.1 ±8.9) mmHg,切断双侧迷走神经后平均动脉压变化最大值为(19.3 ±5.2) mmHg,1 h 后恢复至基础值($P > 0.05$,与切断双侧迷走神经前比较)。各组待血压恢复后,再 icv EM-1 20 μg·kg^{-1}。结果表明,Phen, Prop 和 *L*-NNA 对 EM-1 引起的血压降低无影响, Atro 和切断双侧迷走神经减弱 EM-1 引起的血压降低(表 3)。

Table 3 Effects of vagotomy, iv atropine, phentolamine, propranolol and *L*-NNA on the depressor effect induced by endomorphin-1 (icv, 20 μg·kg^{-1}) in anesthetized rats

Treatment	n	Mean arterial pressure / kPa		
		Baseline value before pretreatment	Baseline value after pretreatment	Maximal change of endomorphin-1
EM-1 after NS	6	94.2 ±4.7	94.5 ±6.2 #	- 20.4 ±1.9
EM-1 after vagotomy	8	97.1 ±8.9	98.0 ±9.3 #	- 4.6 ±1.3 * *
EM-1 after Atro	7	95.2 ±8.8	95.5 ±9.1 #	- 6.0 ±1.7 * *
EM-1 after Phen	7	96.1 ±8.0	96.2 ±7.7 #	- 20.2 ±2.2 *
EM-1 after Prop	7	100.8 ±6.3	101.6 ±6.0 #	- 20.5 ±1.7 *
EM-1 after *L*-NNA	7	96.8 ±7.9	96.5 ±7.3 #	- 20.8 ±3.3 *

$\bar{x}$ ± s. # $P > 0.05$ vs baseline value before pretreatment. * $P > 0.05$, * * $P < 0.01$ vs EM-1 after NS. NS, Naloxone, atropine, phentolamine, propranolol and *L*-NNA was iv injected, and the mean arterial pressure recovered to the baseline value before pretreatment over a 10 - 15 min period ($P > 0.05$). In the bilateral vagotomy group, the mean arterial pressure recovered over 1 h ($P > 0.05$). When the mean arterial pressure recovered, endomorphin-1 (20 μg·kg^{-1}, icv) was injected. Atro: 50 μg·kg^{-1}; Phen: 100 μg·kg^{-1}; Prop: 200 μg·kg^{-1}; *L*-NNA: 25 mg·kg^{-1}

讨 论

本实验 icv 给药方法经组织学检查证实确在侧脑室。免疫组化结果显示[9], EM-1 作为 μ 阿片受体的内源性配体,广泛分布于孤束核、室旁核、隔区、斜角带、终纹床核、僵核、丘脑腹后内侧核、下丘脑后核、导水管周围灰质、蓝斑、伏核和杏仁核等部位,提示其可能在中枢水平调节心血管活动。对麻醉大鼠 icv EM-1 引起剂量依赖、Nx 敏感的血压降低的结果表明, EM-1, 象 β-内啡肽和强啡肽 A 一样,是在中枢环节通过经典阿片受体参与血压调节的重要的内源性阿片肽之一。我们以前的研究[6]表明, iv EM-1 30 nmol·kg^{-1}(约 20 μg·kg^{-1}) 使麻醉大鼠血压变化(- 37.0 ±3.1) mmHg,而 icv 20 μg·kg^{-1} 血压变化(- 21.3 ±3.0) mmHg,是静脉注射的 57.5 %,表明其中枢给药的降血压作用弱于外周给药;而 icv EM-1 引起的血压降低持续时间(16 - 20 min) 显著长于 iv EM-1(4.0 - 6.0 min)。

有报道指出,中枢注射吗啡通过调节孤束核压力反射或兴奋迷走神经而降血压[10,11],中枢注射 DAMGO 通过抑制孤束核压力反射或交感神经活动而降血压[12,13]。icv 或 iv α 受体阻断剂 Phen 和 β 受体阻断剂 Prop 对 icv EM-1 引起的降血压效应无影响,而 icv 或 iv M 受体阻断剂 Atro 或切断双侧迷走神经显著减弱 icv EM-1 引起的降血压效应,推测 icv EM-1 对麻醉大鼠诱发的降血压作用通过中枢 M 受体参与、兴奋迷走神经所致,与吗啡相似;但与中枢肾上腺素受体和交感神经系统无关。iv EM-1 通过作用于血管内皮细胞释放 NO 而降血压[6],但 iv NOS 抑制剂 *L*-NNA 并不影响 icv EM-1 的降血压效应,表明中枢注射 EM-1 的降血压机制与外周注射的机制不完全相同,不能引起血管内皮细胞 NO 的释放。icv 或 iv M 受体阻断剂 Atro 或切断双侧迷走神经对 icv EM-1 引起的血压降低分别减弱 83.4 %, 70.6 %和 77.5 %,并不能完全阻断,暗示可能还有其他机制参与中枢注射 EM-1 引起的血压降低反应。

综上研究表明,icv EM-1 同 iv EM-1 一样,也引起麻醉大鼠血压的显著降低,但作用强度显著低于 iv EM-1,而持续时间显著比 iv EM-1 长;此效应由中枢阿片受体介导、与中枢 M 受体和激活迷走神经有关;而与中枢 α,β 受体和交感神经无关。

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EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF ENDOMORPHIN-1 ON BLOOD PRESSURE IN ANESTHETIZED RATS

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ABSTRACT: **AIM** To observe the effects of intracerebroventricular administration of endomorphin-1 on blood pressure in anesthetized rats and to assess its mechanism. **METHODS** Variations of mean arterial pressure (MAP) were observed after icv injection of endomorphine-1 in rats. The effects of iv or icv of various blockers, naloxone, phentolamine, propranolol, atropine and N^{ω}-nitro-*L*-arginine, on the variation of MAP caused by endomorphin-1 were observed. **RESULTS** Endomorphin-1 was shown to decrease MAP. The decrease in MAP was blocked by naloxone. Pretreatments with icv phentolamine and propranolol showed no effect on the vasodepression induced by icv endomorphin-1. However, pretreatment with icv atropine (25 $\mu g \cdot kg^{-1}$) attenuated the vasodepression. Pretreatments with iv phentolamine, propranolol and N^{ω}-nitro-*L*-arginine showed no effect on the vasodepression induced by icv endomorphin-1. But, pretreatment with iv atropine (50 $\mu g \cdot kg^{-1}$) and bilateral vagotomy attenuated the vasodepression. **CONCLUSION** Intracerebroventricular administration of endomorphin-1 produces vasodepressor response in anesthetized rats, which is mediated by opioid receptor. The vasodepression is associated with the central M cholinoceptor and the excitation of the vagus.

KEY WORDS: endomorphin-1; vasodepression; opioid receptor; central M cholinoceptor