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Research Paper

Tolerance and dependence of edomorphin-1 in rats and possible mechanisms

Weiyan Li*, Sihai Zhu, Hongjun Liu, Jianjun Yang, Jianguo Xu

Department of Anesthesiology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing 210002, Jiangsu Province, China

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Abstract

Objective: To observe the tolerance and the dependence of endomorphin-1 (EM-1) in rats and the possible mechanisms. **Methods:** Sixty Sprague-Dawley rats were randomly allocated into saline, acute EM-1-treated and chronic EM-1-treated groups. The rats were intracerebroventricularly injected with saline, acute EM-1 10 μg/kg 30 min prior to sacrifice, and chronic EM-1 by daily administration at 8:00 A.M. and 15:00 P.M. from 10 μg/kg on the 1st day to 50 μg/kg on the 9th day, respectively. In chronic EM-1-treated group, the median antinociceptive dose (AD_{50}) and the catatonic median effective dose (ED_{50}) were determined by the improved Dixon´s method. Natural withdrawl test was used to assess the dependence of EM-1. Maximal binding capacity (Bmax) and dissociation constant (Kd) of ^{3}H-DAMGO, binding to mu-opioid receptor (MOR) in brain tissue, was measured by Scatchard analysis. Gene expression of MOR was measured by reverse transcription-polymerase chain reaction (RT-PCR). **Results:** Tolerance of the antinociceptic and catatonic effects on the 3rd day (3.1-fold and 1.9-fold) and the 9th day (28.4-fold and 8.5-fold) were observed in chronic EM-1-treated group ($P < 0.05$). Jumping times and withdrawal scores of rats were significantly higher in the chronic EM-1-treated group than those in saline group on the 9th day ($P < 0.05$). Bmax and mRNA expression of MOR in cortex, midbrain and striatum were lower in chronic EM-1-treated group on the 9th day than the other two groups ($P < 0.05$), but Kd had no significant difference ($P > 0.05$). AD_{50}, ED_{50}, Bmax, Kd and gene expression of MOR were recorded. **Conclusion:** EM-1 possesses the tolerance and the dependence. After a long-term treatment, EM-1 down regulates the binding capacity and mRNA of MOR, which somewhat accounts for the dependence.

Keywords: edomorphin-1; tolerance; dependence; rats; mechanisms

INTRODUCTION

Opioid peptides, the ligands for MOR, include exogenous opiates and endogenous opioid peptides [1,2]. EM-1, one of the endogenous opioid peptides, is a newly discovered potent and selective endogenous agonists for MOR [3-5]. Most of the opioid peptides, for example morphine and pethidine, possess the tolerance and the dependence [6,7]. Howerver, it remains unknown if EM-1 possesses the tolerance and the dependence. A prospective randomized control study was designed to observe the tolerance and dependence of EM-1 in rats and the possible mechanisms.

*Corresponding author.

E-mail address: weiyanlee@yahoo.com.cn.

MATERIALS AND METHODS

Animals

The Animal Center of Jinling Hospital provided sixty normal adult male Sprague-Dawley rats according to the National Clean Level and the Ethics Committee of Jinling Hospital approved the present study. The weights of the rats were 180-220 g and they were randomly allocated into saline ($n = 10$), acute EM-1-treated ($n = 10$) and chronic EM-1-treated ($n = 40$) groups. EM-1 was diluted by saline to 500 mg/L (Sagon Co., Shanghai, China).

Drug administration

The rats were injected through the intracerebroventricular cannulation according to Tseng's re-

port[8] with saline(saline group) or acute EM-1 10 μg/kg (acute EM-1-treated group). In the chronic EM-1-treated group, EM-1 was administered daily at 8:00 and 15:00 the dose 10,15,20,25,30,35,40,45, 50 μg/kg on the 1st-9th day, respectively, and the rats were observed on the 1st(n = 10),3rd(n = 10), 6th(n = 10) and 9th(n = 10) day. The volume of the injected solution was the same 20 μl in each rat.

Reaction time of tail-flick

Homeothermic hot water was used as pain source to measure pain reaction with the room temperature 20-22℃. The tail tip of rat was dipped into hot water more than 5 cm. The reaction time of pain from dipping into to withdrawing from water was recorded and the reaction time more than doubled was regarded as the drug having analgesic effect.

Tolerance and dependence of EM-1

After measuring the reaction time of tail-flick of EM-1, the median antinociceptive dose (AD_{50}) and the catatonic median effective dose (ED_{50}) were determined by the improved Dixon's method [9](posttreatment AD_{50}/pretreatment AD_{50} and posttreatment ED_{50}/pretreatment ED_{50}). The natural withdrawl test was used to assess the dependence of EM-1 on the 6th and 9th day. The jumping times were recorded and the withdrawl scores were assessed according to Hellemans' report[10].

Maximal binding capacity (Bmax) and dissociation constant(Kd)

The rats were sacrificed 30 min after the last injection and the brain tissues including cortex, midbrain and striatum were collected. Using DAMGO (D-lactamine 2-methylphenylalanine 4-glycolamine 5-phenol, enkephalin, Sigma-Aldrich Co., USA) as a non-specific control and 3H-DAMGO (Amersham Co., USA) as a radioligand, Scatchard analysis was made to measure Bmax and Kd binding to (MOR) in brain tissue.

Gene expression of MOR

Using β-actin (sense:5′-TAA AGA CCT CTA TGC CAA CAC-3′, antisense:5′-TAA AGC CAT GCC AAA TGT CTC-3′, Institute of Biochemistry, Chinese academy of science,Shanghai,China) as an internal control,MOR mRNA expression (sense:5′-ACC TGG CTC CTG GCT CAA CTT-3′, antisense:5′-TGG ACC CCT GCC TGT ATT TTG-3′[11], Institute of Biochemistry, Chinese academy of science, Shanghai, China) was measured by RT-PCR, and was calculated as β-actin-cDNA/MOR(%).

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed by statistics package for social science (SPSS) of 10.5-version. After a test for homogeneity of related variances, inter-group comparisons were made using one-way analysis of variance(ANOVA) followed by Student-Newman-Keuls test for post-hoc multiple comparisons. P value < 0.05 was considered as statistically significant.

RESULTS

There was no significant difference regarding EM-1 AD50 and EM-1 ED50 before treatment (P > 0.05). Tolerance of the antinociceptic and catatonic effects on the 3rd day(3.1-fold and 1.9-fold) and the 9th day(28.4-fold and 8.5-fold) were observed in the chronic EM-1-treated group(P < 0.05)(***Tab 1***).

Tab 1 **Antinociceptic and catatonic effects of EM-1 in chronic EM-1-treated group** ($\bar{x} \pm s$, n=10)

Time	EM-1 AD_{50}			EM-1 ED_{50}		
	Before treatment (μg/kg)	After treatment (μg/kg)	Degree of tolerance	Before treatment (μg/kg)	After treatment (μg/kg)	Degree of tolerance
1st day	9.3 ± 0.6	13.2 ± 0.8	1.4	10.2 ± 1.4	9.2 ± 2.0	0.9
3rd day	9.1 ± 0.5	28.3 ± 1.0**	3.1	10.2 ± 1.4	19.3 ± 0.9**	1.9
6th day	10.9 ± 2.4	119.9 ± 3.2**	10.9	10.2 ± 1.4	48.3 ± 1.2**	4.8
9th day	10.0 ± 1.9	284.3 ± 12.6**	28.4	10.2 ± 1.4	87.2 ± 3.1**	8.5

Compared with the group before treatment, **P < 0.01

There was no significant dependence of EM-1 on the 6th day(P > 0.05). Jumping times and withdrawal score of rats were significantly higher in chronic EM-1-treated group than those in saline group on the 9th day(P < 0.05)(***Tab 2***).

There was no significant difference regarding Kd between groups(P > 0.05). There was no significant difference regarding Bmax between saline group,

Tab 2 **Jumping times and withdrawal scores in natural withdrawal test** ($\bar{x} \pm s$, n = 10)

Group	Jumping times		Withdrawal scores	
	6th day	9th day	6th day	9th day
Saline	0.0 ± 0.1	0.2 ± 0.4	4.0 ± 2.8	4.0 ± 2.8
Chronic EM-1-treated	0.1 ± 0.3	7.2 ± 1.5**	6.2 ± 0.4	14.5 ± 3.0*

Compared with saline group, *P < 0.05 and **P < 0.01

acute EM-1-treated group and chronic EM-1-treated group on the 1st, 3rd, 6th day ($P > 0.05$) but Bmax of striatum ($P < 0.05$). Bmax and mRNA expression of MOR in cortex, midbrain and striatum were lower in chronic EM-1-treated group on the 9th day than the other two groups ($P < 0.05$) (***Tab 3***).

Tab 3 **Bmax and mRNA expression of brain MOR in chronic EM-1-treated group**

($\bar{x} \pm s$, $n = 10$)

Group	^{3}H-DAMGO Bmax (fmol/mg protein)			MOR mRNA(%)		
	Cortex	Midbrain	Striatum	Cortex	Midbrain(n)	Striatum
Saline	282 ± 33	327 ± 20	402 ± 24	11.0 ± 0.7	11.5 ± 0.5	15.1 ± 0.9
Acute EM-1-treated	304 ± 30	341 ± 22	424 ± 31	12.5 ± 0.8	13.1 ± 1.1	17.4 ± 1.4
Chronic EM-1-treated						
1st day	302 ± 19	336 ± 27	473 ± 26*	12.1 ± 1.0	12.4 ± 0.8	17.6 ± 2.1
3rd day	290 ± 17	315 ± 19	431 ± 21	11.8 ± 0.9	12.0 ± 1.1	15.2 ± 1.5
6th day	241 ± 21	293 ± 24	397 ± 27	10.2 ± 1.3	10.7 ± 1.5	10.7 ± 0.7*
9th day	189 ± 24*	248 ± 20*	295 ± 18**	7.9 ± 1.6*	8.2 ± 1.2*	7.1 ± 1.2**

Compared to saline group, *$P < 0.05$ and **$P < 0.01$

DISCUSSION

EM-1, an endogenous opioid tetrapeptide, reported within the central nervous system, was found having the tolerance and the dependence in the present study. Catatonic effect, which is easily confused with antinociceptic effect, is a tensional and spastic status making pain threshold high when exogenous drug is intracerebroventricularly injected [8]. AD_{50} and ED_{50} of EM-1 before treatment were 9.8±1.6 μg/kg and 10.2±1.4 μg/kg, respectively, which implied that EM-1 is an endogenous opioid peptide and the sample of EM-1 had high purity in the present study.

The increase of AD_{50} and ED_{50} after treatment with EM-1 in chronic EM-1-treated group implied that EM-1 possessed the significant tolerance. On the other hand, the higher jumping times and withdrawal score of rats in chronic EM-1-treated group in natural withdrawl test implied that EM-1 possessed the significant dependence.

Ever since a long time ago, the tolerance and dependence were believed to be comitant[12]. However, in the present study, they were not comitant, which is also reported recently by more and more documents [13,14]. The difference of the occurrence of the tolerance and dependence showed that EM-1, as an endogenous opioid peptide, perhaps was more adaptive to MOR.

The decrease of Bmax and no change of Kd implied that the binding sites of MOR were lower after chronically treated with EM-1. The decreased gene expression of MOR showed that MOR decreased after being chronically treated with EM-1. The main mechanisms of MOR downregulation included the internalization and the block of gene expression[13-15]. Ronnekleiv *et al*[16] and Magendzo *et al*[17] also found that the mRNA expression of MOR decreased in basal ganglia of cavies after chronically treated with morphine.

The tolerance occurred on the 3rd day, and the dependence, the downregulation of Bmax and MOR gene expression occurred on the 9th day, which implied the downregulation of Bmax and MOR gene expression accounted for the dependence. Tao PL and coworkers[18] reported that the downregulation of MOR in chronic enkephalin treatment didn't concern with the tolerance. The similar conclusions were also drawn by Chaturvedi[19] and Liu-Chen[20].

From the results of the present study, we concluded that EM-1 possesses the tolerance and the dependence, and the tolerance occurs before the dependence. After a long-term treatment, EM-1 down regulates the binding capacity and mRNA of MOR, which somewhat accounts for the dependence but not for the tolerance.

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