高压液相色谱法

目的：研究苯巴比妥(Phe)诱导肝细胞增殖和阻断肝细胞凋亡的作用是否与肝内局部体液因素有关. 方法：观察 Phe 诱导增殖的小鼠肝提取物(PMLE)及其加热提取物 (PMLE-95)对小鼠肝有无 Phe 样作用；经胰蛋白酶、RNA 酶或 DNA 酶预处理后，其活性是否消失. 高效液相色谱分析增殖肝与非增殖肝加热提取物(NMLE-95)的组份差异. 结果：PMLE-95 使正常肝细胞增殖，阻断撤除 Phe 引起的小鼠肝细胞凋亡；胰蛋白酶处理后，该作用消失. HPLC 分析，PMLE-95 比 NMLE-95 多一个主峰. 结论：Phe 诱导小鼠肝细胞增殖和阻断肝细胞凋亡的作用与肝内局部体液因素有关，该因素是一种蛋白质或肽类物质.

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Levels of immunoreactive dynorphin A_{1-13} during development of morphine dependence in rats[1]

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KEY WORDS dynorphin; morphine; opioid-related disorders; radioimmunoassay; substance withdrawl syndrome

AIM: To study the relationship between the levels of immunoreactive dynorphin A_{1-13} (ir-dynorphin A_{1-13}) and the degree of morphine dependence. **METHODS:** The levels of ir-dynorphin A_{1-13} in discrete brain regions, spinal cord, and plasma in rats were determined by radioimmunoassay, and the degree of morphine dependence was assessed by scoring withdrawal signs on d 3, d 6, and d 12. **RESULTS:** Morphine injection sc decreased the levels of ir-dynorphin A_{1-13} in spinal cord, pituitary, and plasma. The levels of ir-dynorphin A_{1-13} in hippocampus and hypothalamus were increased. No changes in cortex, midbrain, cerebellum, pons, and medulla were observed. With continuous injection of morphine, withdrawal signs scores were increased on d 6, but there was no difference between the scores of d 6 and d 12. **CONCLUSION:** The changes of the levels of endogenous ir-dynorphin A_{1-13} in pituitary, spinal cord, and plasma were compatible with the degree of morphine dependence.

Dynorphin A_{1-13} can ameliorate the signs of opiate withdrawal and suppress the expression of opiate tolerance in morphine-dependent mice[1,2], rats[3], monkey[4], and heroin addicts[5]. Ir-dynorphin A_{1-13} plays an important role during the induction of tolerance to and dependence on morphine in rats, and it is possible that ir-dynorphin A_{1-13} levels in spinal cord and some brain regions were decreased, so that the administration of exogenous dynorphin A_{1-13} restored the levels and thereby attenuated withdrawal signs. However, the results obtained were unlike[6,7] and during the development of dependence on morphine the dynamic changes of ir-dynorphin A_{1-13} levels in rats and its relationship with the degree of dependence have not ever been reported. The present experiments were to study the relationship between the levels of immunoreactive dynorphin A_{1-13} and the degree of morphine dependence in rats.

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MATERIALS AND METHODS

Rats Sprague-Dawley ♂ rats, weighing 200 g ± *s* 18 g (Sino-British Sippr/BK Lab Animal Ltd, Shanghai, Grade Ⅱ, Certificate No 02-49-2) were divided into 4 groups: 1) control; 2) 3-d morphine injection; 3) 6-d morphine injection; 4) 12-d morphine injection

Materials Morphine hydrochloride (Qinghai Pharmaceutical Factory); naloxone hydrochloride (Beijing Four-Ring Pharmaceutical Factory); Dynorphin A_{1-13} (Sigma Chemical Co); antiserum to dynorphin A_{1-13} (Department of Neurobiology, Second Military Medical University). Other chemicals were of AR grade.

Induction of physical dependence on morphine and scoring of withdrawal signs To render the physically dependent rats on morphine, the rats were injected sc morphine hydrochloride tid at 5 $mg \cdot kg^{-1}$ increment until d 3, d 6, and d 12. The initial dose was 30 $mg \cdot kg^{-1} \cdot d^{-1}$. When the dose reached 150 $mg \cdot kg^{-1} \cdot d^{-1}$ on d 9, it was maintained until d 12. Control rats were similarly injected with saline.

The degree of physical dependence on morphine was assessed[3] by scoring withdrawal signs precipitated by ip naloxone 4 $mg \cdot kg^{-1}$ for 6 h after the last sc morphine solution or saline. Following withdrawal signs 0 – 60 min after naloxone injection were scored: body weight loss, rearing, abdominal contraction, wet-dog shaking, diarrhea, teeth-chattering, swallowing, irritability, ptosis, abnormal posture, writhing, salivation, and penile discharge.

Extraction of ir-dynorpnin A_{1-13} from tissues Six hours after the last sc morphine or saline, rats were decapitated. Plasma was collected in polypropylene tube containing aprotinin 50 μL and sodium heparin 10 μL. Brain, spinal cord, and pituitary gland were boiled in saline for 10 min, and the brains were dissected into 7 regions: cerebellum, cortex, hippocampus, hypothalamus, midbrain, pons and medulla, and striatum[8]. The tissues were transferred into a polypropylene tube containing 1 mL HAc (0.1 $mol \cdot L^{-1}$) and homogenized with a polytron homogenizer at 4 ℃, and then 1 mL NaOH (0.1 $mol \cdot L^{-1}$) was added to neutralize the solution, after centrifugation at 4000 × *g* at 4 ℃ for 20 min, the supernatants were stored at – 30 ℃ until assay[9].

RIA of ir-dynorphin A_{1-13}[9,10] Antiserum was used at a final dilution of 1:25 000 for 35 % binding rate of ^{125}I-dynorphin A_{1-13}. The mixture contained 100 μL of sample or standard peptide, 100 μL of antiserum, and 100 μL of tracer (150 $MBq \cdot L^{-1}$). The solution was incubated at 4 ℃ for 24 h. The separation of bound and free antigen was carried out by addition of sheep antirabbit antiserum 500 μL and incubated for 1 h. After centrifugation, the radioactivity in the pellet was counted with Gamma 5500 counter (Beckman Instruments Inc, USA). Protein concentrations in tissue samples were determined[11] in duplicate.

Statistic analysis Data were expressed as $\bar{x} \pm s$ and evaluated by Student-Newman-Keuls test.

RESULTS

Withdrawal signs In morphine-dependent rats the pattern of behavior induced by ip naloxone 4 $mg \cdot kg^{-1}$ showed a characteristic duration-dependent development. With consecutive injections of morphine, weight loss became severe, and the frequency of rearing, abdominal contraction, and wet-dog shaking were increased ($P < 0.05$), but after 6-d morphine injection, the score of withdrawal signs was not increased any more. For the checked withdrawal signs, diarrhea, teeth-chattering, and irritability were observed even in rats of 3-d morphine injection, but ptosis, abnormal posture, and salivation were seldom seen. In rats of 6-d and 12-d morphine injection, almost all signs were noticed within 0 – 60 min after naloxone precipitation (Tab 1).

Tab 1. Scoring of withdrawal signs in rats dependent on morphine. $n = 7$ rats, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* 3-d morphine injection. $^gP > 0.05$ *vs* 6-d morphine injection. Withdrawal syndrome was precipitated by naloxone ip 4 $mg \cdot kg^{-1}$.

Days of injection	Morphine/ $mg \cdot kg^{-1} \cdot d^{-1}$	Withdrawal syndrome score
0	0	2.9 ± 1.6
3	60	16.5 ± 2.8^c
6	105	35.1 ± 2.8^f
12	150	37.5 ± 1.9^g

Effect of morphine injection on levels of ir-dynorphin A_{1-13} Of all the tissues examined, the highest level of ir-dynorphin A_{1-13} was found in the pituitary gland, the levels of other dissections of brain and spinal cord were decreased in the following order: hypothalamus, spinal cord, midbrain, pons and medulla, hippocampus, cortex, striatum, and cerebellum (Tab 2).

Throughout 12 d of morphine sc the levels of ir-dynorphin A_{1-13} in cerebellum, midbrain, cortex, striatum and pons, and medulla were not changed. However, a decrease in dynorphin A_{1-13} level was observed in spinal cord (48.7 %, $P < 0.01$ *vs* control) and pituitary (32 %, $P < 0.01$ *vs* control) after 6-d morphine sc. The level of ir-dynorphin A_{1-13} in plasma was decreased to 69.9 % of control level after 3 d and 36.4 % after 6 d. In all brain regions investigated only the levels in hippocampus and hypothalamus were increased. Compared to the levels of ir-dynorphin A_{1-13} on d 6, the levels after 12-d morphine sc showed no difference, remaining at a relatively constant levels. The changes of ir-dynorphin A_{1-13} levels in pituitary, spinal cord, and plasma were accordant with severity of naloxone-precipitated withdrawal syndrome.

DISCUSSION

The functional role of the dynorphin A in morphine dependence mechanism is not well understood. Formerly, the effect of morphine injection on the levels of ir-dynorphinA_{1-13} was all investigated at a fixed time point, and time-course changes have not ever reported. The major findings of this study were that morphine injection did not decrease the levels of dynorphin A_{1-13} constantly, and rats were rendered strongly dependent on morphine by 6-d morphine sc thrice per day, further consecutive administration of morphine did not lead to an increase in the score of withdrawal signs precipitated by naloxone. This dynamic change was accordant with the trend of dynamic change in the levels of ir-dynorphin A_{1-13} in plasma, spinal cord, and pituitary. Our study demonstrated that to induce tolerance and dependence on morphine in rats, sc morphine thrice per day for 6 d is a suitable procedure. The relationship between the severity of withdrawal signs and the levels of ir-dynorphin A_{1-13} in plasma, spinal cord, and pituitary suggest that endogenous ir-dynorphin A_{1-13} may play an important role in suppressing the withdrawal syndromes of rats. However, our study suggested that the changes in the levels of ir-dynorphin A_{1-13} in morphine-dependent rats were region specific and were not all in the same direction. It is possible that the regulation of ir-dynorphin A_{1-13} in the brains is region-specific.

In conclusion, rats dependent on morphine were rendered by 6-d morphine sc thrice per day, the dynamic changes of ir-dynorphin A_{1-13} in pituitary, spinal cord, and plasma were compatible with the severity of withdrawal signs in morphine dependent rats. The decrease of the

Tab 2. The content of ir-dynorphin A_{1-13} in brain regions, spinal cord, and plasma of rats dependent on morphine. $n = 7$ rats, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* 3-d morphine sc. $^gP > 0.05$ *vs* 6-d morphine sc. Administration procedure was the same as Tab 1.

Tissues	Content of ir-dynorphin A_{1-13} (μg/g protein)		
	Control	3-d injection	6-d injection	12-d injection
Pituitary	48.12 ± 2.81	36.74 ± 1.76^c	30.68 ± 1.85^f	32.25 ± 1.25^g
Cortex	0.70 ± 0.11	0.69 ± 0.08^a	0.75 ± 0.11^d	0.72 ± 0.06^g
Hippocampus	0.83 ± 0.10	1.06 ± 0.12^b	1.37 ± 0.17^e	1.53 ± 0.24^g
Striatum	0.45 ± 0.08	0.49 ± 0.04^a	0.50 ± 0.07^d	0.47 ± 0.07^g
Hypothalamus	2.31 ± 0.16	2.64 ± 0.24^b	2.89 ± 0.63^d	2.91 ± 0.40^g
Midbrain	1.04 ± 0.13	1.20 ± 0.27^a	1.17 ± 0.26^d	1.08 ± 0.13^g
Cerebellum	0.08 ± 0.02	0.08 ± 0.02^a	0.08 ± 0.02^d	0.07 ± 0.01^g
Pons and medulla	0.96 ± 0.11	0.87 ± 0.08^a	0.92 ± 0.13^d	0.96 ± 0.09^g
Spinal cord	1.17 ± 0.06	0.82 ± 0.12^b	0.60 ± 0.09^e	0.64 ± 0.13^g
Plasma ($\mu g \cdot L^{-1}$)	0.55 ± 0.04	0.38 ± 0.02^c	0.20 ± 0.04^f	0.26 ± 0.05^g

levels of ir-dynorphin A_{1-13} in pituitary, plasma, and spinal cord may contribute to the withdrawal syndrome in morphine-dependent rats.

REFERENCES

1 Tulunay FC, Jen MF, Chang JK, Loh HH, Lee NM. Possible regulatory role of dynorphin on morphine- and beta-endorphin-induced analgesia. J Pharmacol Exp Ther 1981; 219: 296 - 8.

2 Takemori AE, Loh HH, Lee NM. Suppression by dynorphin A-(1 - 13) of the expression of opiate withdrawal and tolerance in mice. Eur J Pharmacol 1992; 221: 223 - 6.

3 Green PG, Lee NM. Dynorphin A-(1 - 13) attenuates withdrawal in morphine-dependent rats: effect of route of administration. Eur J Pharmacol 1988; 145: 267 - 72.

4 Aceto MD, Dewey WL, Chang JK, Lee NM. Dynorphin A-(1 - 13) effects in non-tolerant and morphine-dependent rhesus monkeys. Eur J Pharmacol 1982; 83: 139 - 42.

5 Wen HL, Ho WKK. Suppression of withdrawal of symptoms by dynorphin in heroin addicts. Eur J Pharmacol 1982; 82: 183 - 6.

6 Nylander I, Sakurada T, Grevens PL, Terenius L. Levels of dynorphin peptides, substance P and CGRP in the spinal cord after subchronic administration of morphine in the rats. Psychopharmacol 1991; 30: 1219 - 23.

7 Nylander I, Vlaskivka M, Terenius L. The effect of morphine treatment and morphine withdrawal on the dynorphin and enkephalin systems in Sprague-Dawley rats. Psychopharmacol 1995; 118: 391 - 400.

8 Bhargava HN, Gulati A. Modification of brain and spinal cord dopamine D_1 receptors labeled with ^{3}H-SCH23390 following morphine withdrawal from tolerant and physically dependent rats. J Pharmacol Exp Ther 1990; 252: 901 - 7.

9 Ghazaroesian VE, Chavkin C, Goldstein A. A specific radioimmunoassay for the novel opioid peptide dynorphin. Life Sci 1980; 27: 75 - 86.

10 Wang CH, Zhu YX, Wu CR, Song CY, Lin BC. Radioimmunoassay for dynorphin A1 - 13. Acta Pharmacol Sin 1987; 8: 494 - 7.

11 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265 - 75.

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大鼠吗啡依赖形成过程中免疫活性强啡肽 A_{1-13} 的含量[1]

R917

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关键词 强啡肽类；吗啡；阿片类有关的紊乱；放射免疫测定；物质撤除综合征

目的：研究吗啡依赖大鼠组织内免疫活性强啡肽 A_{1-13} 含量的动态变化及其与依赖程度的关系。**方法**：用纳洛酮催促的戒断症状评分测定吗啡依赖程度，用放射免疫测定组织内强啡肽 A_{1-13} 水平。**结果**：在 3 - 6 天给药期内，吗啡可进行性降低脊髓、垂体、血浆内免疫活性强啡肽 A_{1-13} 水平，升高海马及下丘脑的强啡肽 A_{1-13} 水平，继续给药至 12 天，各组织内免疫活性强啡肽 A_{1-13} 水平不再有显著性变化。**结论**：吗啡依赖形成过程中脊髓、垂体及血浆内强啡肽 A_{1-13} 呈进行性降低，其趋势与吗啡依赖程度一致。