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Effects of morphine and naloxone on proliferation of lymphocytes *in vitro*[1]

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AIM: To study the effects of morphine on different lymphocytes and the influence of naloxone. **METHODS**: The proliferation rates of unmature, resting, and activated T-lymphocytes and B-lymphocytes were determined under various concentrations of morphine with or without naloxone *in vitro*. **RESULTS**: Morphine $1\times10^{-10}-1\times10^{-6}$ mol L^{-1} enhanced concanavalin A (Con A)-induced splenic T-cell proliferation, and 1 μmol L^{-1} enhanced lipopolysaccharide (LPS)-induced splenic B-cell proliferation. Naloxone, which *per se* enhanced the T-cell proliferation, blocked the enhancing effects of morphine. Morphine ($1\times10^{-10}-1\times10^{-5}$ mol L^{-1}) had no influence on the proliferation of resting splenocytes and Con A-induced thymus cells. Morphine 1 mmol L^{-1} inhibited the proliferation of resting, LPS-induced splenocytes, and Con A-induced splenic and thymus cells. These inhibiting effects were not blocked by naloxone (50 μmol L^{-1}). **CONCLUSION**: Stimulating effect of morphine on activated T- and B-cells were mediated by opioid receptors and different opioid receptors existed during the differentiation and activation of lymphocytes. The inhibitory effects of morphine (1 mmol L^{-1}) were not mediated by opioid receptors.

KEY WORDS morphine; naloxone; opioid receptors; T-lymphocytes; B-lymphocytes; concanavalin A; lipopolysaccharides; cultured cells

Morphine addiction is accompanied by increased infections due to immunosuppression by drug abuse[1]. This morphine-induced immunosuppression is partially mediated by the activation of adrenal cortex via hypothalamo-pituitary-adrenal axis[2] and/or the central opioid system of the periaqueductal gray[3]. A review[4] on the effects of opiates on the immune system concluded that the case for specific opioid binding was unconvincing. However the preponderant data reported suggests that opiate receptors exit on the surface of at least some of the cells of the immune system. The purpose of our work is to study the direct proliferative effects of morphine on different kinds of lymphocytes under different conditions *in vitro* and the relationship with opioid receptor antagonist naloxone.

MATERIALS AND METHODS

Reagents Morphine was produced by Qinhai Pharmaceutics and purified by Dr YIN Dun-Xiang, Department of Synthetic Chemistry of our Institute. Trypan blue, produced by the Third Shanghai Reagent Factory, was dissolved in 0.5 % saline. Naloxone, concanavalin A (Con A), and lipopolysaccharides (LPS) from *E coli* were from Sigma Co and [^{3}H]TdR was from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. The medium RPMI-1640 (Gibco Co) was supplemented with HEPES buffer 10 mmol L^{-1}, penicillin 100 kU L^{-1}, streptomycin 0.1 g L^{-1}, *L*-glutamine 2 mmol L^{-1}, 2-mercaptoethanol 50 mol L^{-1}, and 10 % fetal bovine serum (FBS), pH 7.2.

Mice Inbred ICR mice, ♀, 3 months old, 18—

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22 g were purchased from Shanghai Animal Center, Chinese Academy of Sciences.

Proliferation assay Thymus or spleen was torn and passed through a stainless mesh (40 μm pores). Following 2 washes with RPMI 1640, the cell suspension was adjusted to a final concentration of 4×10^6 cells L^{-1} in RPMI 1640. The cell suspension (100 μL) was added to 96-well microtiter plates with or without mitogen (Con A 5 mg L^{-1} or LPS 6.25 mg L^{-1}). Add medium to 200 μL/well. After various concentrations of morphine incubated with or without naloxone (50 mol L^{-1}) at 37 ℃ under 5 % CO_2 for 66 h, [^{3}H] TdR (9.25 kBq/well) was added and the plates were incubated for another 6 h, then harvested onto glass fiber filter. The lymphocyte proliferation was expressed as dpm/2×10^5 cells.

Lymphocyte viability assay After lymphocytes were incubated with morphine 0.1, 1 mmol L^{-1} with or without mitogen for 24, 48, and 72 h, Trypan blue exclusion was used to count 200 cells and calculated the ratio of cell viability.

RESULTS

Effects of morphine on resting and activated spleen lymphocytes Con A-induced spleen T-lymphocyte proliferation was enhanced by morphine $1 \times 10^{-10} - 1 \times 10^{-6}$ mol L^{-1}. LPS-induced B-lymphocyte proliferation was enhanced only by morphine 1 μmol L^{-1}. The proliferation of nonstimulated lymphocytes was not influenced by morphine $1 \times 10^{-10} - 1 \times 10^{-4}$ mol L^{-1}. Morphine 1 mmol L^{-1} depressed both the non-stimulated and stimulated spleen lymphocyte proliferation (Tab 1).

Tab 1. Effects of morphine on resting, Con A (5 mg L^{-1})- and LPS (6.25 mg L^{-1})-induced splenocyte proliferations *in vitro* ([^{3}H]TdR uptake). $n = 4$ wells for 1 homogenate (pooled from 10 mice), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control group.

Morphine/ mol L^{-1}	Con A/ $10^{-3} \times$dpm	LPS/ $10^{-3} \times$dpm	Without mitogen/ $10^{-3} \times$dpm
0	12±2	15±1	3.0±0.5
10^{-10}	21±6^b	13±1^a	3.0±0.3^a
10^{-9}	28±8^c	13±2^a	3.0±0.4^a
10^{-8}	37±2^c	14±2^a	2.9±0.2^a
10^{-7}	30±5^c	13±1^a	3.3±0.4^a
10^{-6}	23±4^c	18±1^c	3.4±0.4^a
10^{-5}	19±2^a	17±2^a	3.6±0.5^a
10^{-4}	8±3^a	14±2^a	2.7±0.2^a
10^{-3}	1±0^c	3±1^c	0.7±0.1^c

Influence of naloxone on morphine effect on activated splenocyte Naloxone (50 μmol L^{-1}) stimulated the Con A-activated T-cell proliferation, antagonized the promotive effect of morphine on the activated T- and B-cell proliferation, but had no influence on the inhibitory effect of morphine 1 mmol L^{-1} on the T- and B-cell proliferation (Tab 2).

Effect of morphine on Con A stimulated thymus lymphocytes The Con A-induced

Tab 2. Effect of morphine and naloxone (50 μmol L^{-1}) on Con A (5 mg L^{-1})-, LPS (6.25 mg L^{-1})-induced splenocyte proliferations *in vitro* ([^{3}H]TdR uptake). $n = 4$ wells for 1 homogenate (pooled from 10 mice), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control group.

Morphine mol L^{-1}	Con A/$10^{-3} \times$dpm		LPS/$10^{-3} \times$dpm	
	−naloxone	+naloxone	−naloxone	+naloxone
0	37±5	52±5^c	6.6±1.3	6.5±1.4
10^{-8}	51±3^c	42±10^a	5.2±0.8^a	5.4±0.9^a
10^{-7}	48±3^c	45±6^a	6.0±0.7^a	5.3±0.3^a
10^{-6}	52±6^b	43±9^a	10.2±0.1^c	4.8±0.8^a
10^{-5}	52±1^a	43±3^a	5.4±0.4^a	5.3±0.5^a
10^{-3}	12±2^c	9±4^c	3.4±0.7^c	2.2±0.1^c

thymus T-cell proliferation was not obviously enhanced by morphine at $1\times10^{-10}-1\times10^{-5}$ mol L^{-1}, morphine 0.1—1 mmol L^{-1} inhibited the thymus lymphocyte proliferation (Fig 1), just like in the case of spleen lymphocyte proliferation.

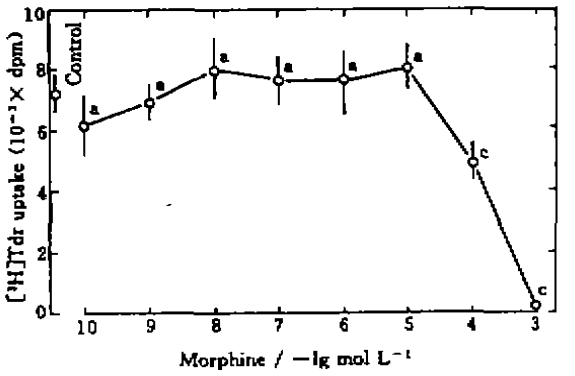

Fig 1. Effect of morphine on Con A (5 mg L^{-1})-induced thymus lymphocyte proliferation *in vitro* ([³H]TdR uptake). $n=4$ wells for 1 homogenate (pooled from 10 mice), $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* control group.

Effect of morphine on lymphocyte viability The ratios of viability of lymphocytes cultured with morphine 1 mmol L^{-1} after 24, 48, and 72 h were all >95 %.

DISCUSSION

Our work demonstrated that morphine at low concentration stimulated the proliferation of Con A-activated T-cells (1×10^{-10} — 1×10^{-6} mol L^{-1}) and LPS-activated B-cells (1 μmol L^{-1}), and the stimulating effects were blocked by naloxone (50 μmol L^{-1}), an opioid receptor antagonist. This suggests that the stimulating effect is mediated by the μ opioid receptors on lymphocytes.

It is worth to point out that the spleen T-cell is more sensitive to morphine than B-cell. This supports the theory that the effect of morphine on B-cell is mediated by its effect on T-cell and/or macrophages but not directly[5].

Morphine directly inhibited both thymus and spleen lymphocytes at millimolar concentrations, which can be reached by high dose of morphine used in evaluating its effects on immunofunction[6]. This suggested that a direct effect of morphine on lymphocyte attribute to immunosuppression of morphine abuser.

Morphine ($1\times10^{-10}-1\times10^{-5}$ mol L^{-1}) had no direct influence on the proliferation of resting splenocytes and Con A-induced unmature thymus T-lymphocytes, suggesting that opioid receptors might be developed during the differentiation and activation of lymphocytes.

In conclusion, morphine directly affected lymphocytes through opioid receptors and non-opiate receptors. Morphine at high concentration inhibited lymphocyte proliferation through the non-opiate receptor pathway and at low concentration stimulated the lymphocyte proliferation through the opiate receptor on lymphocytes.

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吗啡及纳洛酮对淋巴细胞体外增殖的作用[1]

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目的：研究吗啡对不同淋巴细胞增殖的作用及纳洛酮的影响. **方法**：观察吗啡对未成熟的、静止的及活化的脾脏淋巴细胞体外增殖影响及纳洛酮的阻断作用. **结果**：吗啡（1×10^{-10}—1×10^{-6} mol L^{-1}）能增加 Con A 诱导的 T-细胞的增殖，1 μmol L^{-1}还能促进 LPS 诱导的 B-细胞的增殖. 同时这些增强作用都能被纳洛酮 50 μmol L^{-1}阻断. 纳洛酮单独亦能促进活化 T-细胞的增殖. 而吗啡 1×10^{-10}—1×10^{-5} mol L^{-1}对静止的脾脏淋巴细胞及 Con A 活化的胸腺淋巴细胞的增殖都无影响. 但是吗啡 1 mmol L^{-1}能广泛抑制静止的、LPS 活化的脾脏细胞及 Con A 活化的胸腺，脾脏淋巴细胞增殖，且都不能被纳洛酮阻断. **结论**：吗啡对活化 T-和 B-细胞的促进作用是由细胞表面的阿片受体介导的，此阿片受体随着淋巴细胞的成熟和活化而变化. 而吗啡1 mmol L^{-1}对淋巴细胞增殖的抑制作用却不是由经典的阿片受体介导的.

关键词 吗啡；纳洛酮；阿片受体；T-淋巴细胞；B-淋巴细胞；刀豆蛋白 A；脂多糖；培养的细胞

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Platelet adhesion to cultured bovine cerebral microvascular endothelial cells by stimulation of platelet activating factor and antagonism of drugs

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AIM: To study platelet activating factor (PAF) stimulating the platelets to adhere to cultured bovine cerebral microvascular endothelial cells (CMEC) and the inhibitory effect of triazelodiazepine (WEB), 1,5-bis-(3,4-dimethoxyphenyl)-tetrahydro-(4H)-pyran (DMPP), tetrandrine (Tet). **METHODS**: The platelets adhesion to CMEC and the inhibitory effect of drugs were investigated by [^{3}H]adenine labeling of rabbit blood platelet. **RESULTS**: The platelet adhesion to CMEC was increased by 36 % *vs* control after CMEC was stimulated with PAF 10 nmol L^{-1} for 25 min. WEB 0.1, 1, 10 mmol L^{-1} or DMPP 0.1, 1, 10 mmol L^{-1} or Tet 0.1, 1, 10 mmol L^{-1} inhibited the PAF stimulating platelet adhesion to CMEC by 5.4 %, 16.3 %, 20.1 %; 13.7 %, 19.4 %, 22.4 %; and 5.5 %, 23.1 %, 32.6 %, respectively. **CONCLUSION**: DMPP and Tet inhibited the PAF action in cerebral vascular system.

KEY WORDS platelet activating factor; vascular endothelium; platelet adhesiveness; triazoles; tetrandrine; pyrans

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