Evidence for an inhibitory role of β-endorphin and other opioids on human total T rosette formation[1]

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Summary. β-endorphin, met-enkephalin, leu-enkephalin and morphine significantly inhibit rosette formation between human T lymphocytes and sheep red blood cells. This effect is completely reversed by naloxone, a specific antagonist, while naloxone by itself does not influence rosette formation. A further link between the immune system and the neuroendocrine system is suggested.

In addition to central analgesic effects, exogenous (morphine) and endogenous (endorphins) opioids have been shown to exhibit a wide range of pharmacological and physiological activities including those exerted on the immune system. Morphine exerts an interferon-decreasing activity in the mouse[2], and this effect can be regulated by naloxone[3]. A transport system for benzomorphans in leukocytes has been characterized[4], alpha-endorphin-like immunoreactivity has been detected in plasma cells of the canine colonic mucosa[5], high molecular weight immunoreactive β-endorphin has been identified as a fragment of human IgG[6], and human lymphocyte production of endorphin-like substances has been shown[7]. Non-opiate receptors for β-endorphin have been recognized on cultured human lymphocytes[8] and on the terminal complex of human complement[9], while β-endorphin has a potentiating effect, not reversed by naloxone, on rat splenic lymphocyte proliferative responses[10]. Finally, opiate receptors for morphine, dextromoramide and met-enkephalin have been demonstrated on human T lymphocytes[11,12]. The aim of this paper is to investigate the effects of β-endorphin, an opiate released from the pituitary into the blood under physical or emotional stress[13-15] and during parturition[16], on the total T rosette formation of human lymphocytes, and to compare these effects with those of different synthetic and natural analogues.

Materials and methods. The technique for separation of lymphocytes, obtained from normal donors, has already been described[17]. After separation, the cells were washed and suspended at a final concentration of 4×10^6/ml in RPMI 1640 containing the following substances: 10^{-9} M morphine chlorhydrate (Angelini S.p.A.), 10^{-9} M met- or leu-enkephalin (Paesel GmbH and Co., Frankfurt), 10^{-9}, 10^{-10}, 10^{-11} and 10^{-12} M β-endorphin (Paesel GmbH and Co., Frankfurt), 10^{-7} M naloxone chlorhydrate (Narcan^R, Crinos S.p.A.). After 60 min of incubation at 37 °C with each of the substances, the cells were washed and assayed for total T rosette formation as previously reported[18]. Experiments with simultaneous incubation for 60 min at 37 °C of the cells with each of the opiates (10^{-9} M) and their specific antagonist, naloxone (10^{-7} M), were also performed.

The statistical evaluation of the data to compare the results with those from controls (no substance), was performed with the paired Student t-test.

Table 1. Effects of 60 min incubation at 37 °C of human lymphocytes (20 donors) with different dilutions of β-endorphin, ranging from 10^{-12} to 10^{-9} M, on total T rosette formation

β-endorphin concentration (M)	0 (baseline levels)	10^{-12}	10^{-11}	10^{-10}	10^{-9}
Mean ± SD	60.1 ± 6.6	56.9 ± 11.1*	49.5 ± 5.7***	47.4 ± 9.4**	47.1 ± 12.1**

* not significant when compared to baseline levels; ** $p < 0.001$ when compared to baseline levels; *** $p < 0.001$ when compared to 10^{-12} M. A significant decrease of total T rosettes percentage is detected with β-endorphin concentrations ranging from 10^{-11} to 10^{-9} M. When a concentration of 10^{-12} M is used, a slight and not significant reduction of T rosettes is found.

Table 2. Effects of morphine, β-endorphin, met-enkephalin and leu-enkephalin on T rosette formation (6 donors, mean ± SD)

Opiate added	Concentration (M) 0	10^{-9}
Morphine	62.33 ± 5.6	45.16 ± 5.2* (27.5%)
β-endorphin	62.33 ± 5.6	47.66 ± 4.4* (23.5%)
Met-enkephalin	62.33 ± 5.6	46.50 ± 8.0* (25.4%)
Leu-enkephalin	62.33 ± 5.6	49.00 ± 4.3* (21.4%)

* $p < 0.001$; in brackets the percent decay induced by the different opiates compared to the baseline levels (no substance).

Table 3. Reversibility by naxolone of the effects of different opiates on T rosette formation (3 donors)

Opiate added (10^{-9} M)		In presence of 10^{-7} M naloxone
None	63.0 ± 2.6	61.0 ± 2.6*
Morphine	43.6 ± 3.2	65.3 ± 2.8***
β-endorphin	50.0 ± 2.6	64.3 ± 5.7**
Met-enkephalin	44.3 ± 2.9	59.3 ± 1.1***
Leu-enkephalin	48.3 ± 3.2	61.3 ± 3.2**

Results are expressed as mean values ± SD. * not significant; ** $p < 0.05$; *** $p < 0.02$. The presence of naloxone (10^{-7} M) in the incubation mixtures completely abolishes the inhibitory effects of opioid substances (10^{-9} M) on total T rosette formation; naloxone by itself does not modify percentage of T rosettes.

Results and discussion. Table 1 shows that β-endorphin interacts with human lymphocytes and significantly reduces total T rosette formation with a dose-dependent effect. However, when β-endorphin is used at a concentration (10^{-12} M) comparable to that in normal human plasma under physiological conditions[13-16], a slight and not significant decrease of T rosettes is found. Maximum decay is detected when passing from 10^{-12} to 10^{-11} M. It is worth noting that the latter concentration can be reached in humans under physical or emotional stress and during parturition[13-16]. When β-endorphin concentration is increased up to 10^{-9} M, no further significant reduction of T rosettes is detected when compared to 10^{-11} M, thus indicating that not all T lymphocytes can be influenced by β-endorphin in rosette formation with sheep red blood cells.

Table 2 shows that morphine, met- and leu-enkephalin are also capable of interacting with T lymphocytes, resulting in a reduction of total T rosettes. Such reduction is comparable with that obtained using β-endorphin at the same concentration (10^{-9} M).

The specificity of the effects of β-endorphin and other opiates on T rosette formation is proven by a complete reversibility in the presence of naloxone, a specific antagonist of opiates, while naloxone per se is not effective (table 3).

Our results give suggestive evidence that a subpopulation of T

lymphocytes bear opiate receptor sites for β-endorphin and for some synthetic and natural analogues, and support the concept that opioids can be involved in the regulation of lymphocyte activity[10,19]. This finding, and the well-known existence of a subpopulation of human T lymphocytes which react with an heteroantiserum to human brain[20], provides further evidence of close links between the immune system and the central nervous system.

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Potential for rat plague from nonencapsulated variants of the plague bacillus (*Yersinia pestis*)

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Summary. Potentials for oral and flea-borne transmission of nonencapsulated *Y.pestis* were demonstrated when 45% of rats that consumed infected meat died of plague and 22% of the rats that died of plague had bacteremia.

Nonencapsulated (F1−) *Yersinia pestis* are plague bacilli that lack the ability to produce an envelope containing fraction 1 virulence antigen. Nevertheless, infections with certain F1− *Y.pestis* can cause fatal disease in man[2] and laboratory mice[3]. However, laboratory rats that have a high susceptibility to plague from encapsulated (F1+) *Y.pestis* have appeared to be resistant to fulminating disease from F1− organisms, although chronic infections with F1− bacilli occur. As outbreaks of plague in human populations are frequently associated with rat plague, any resistance to disease in rats is significant because it reduces the danger of plague for humans. Resistance results in fewer rats dying and, thus, fewer infected carcasses and hostless fleas to transmit the disease to people. To confirm such resistance in rats, F1− *Y.pestis* of high virulence for mice were inoculated into rats. In addition, the possibility that large infective doses of F1− bacilli might overcome any resistance to disease was examined by feeding infected material to rats.

Materials and methods. F1− *Y.pestis* were isolated on blood agar from the abdominal bubo of a vaccinated rat that died 541 days after challenge with the virulent F1+ *Y.pestis* strain 195/P[4]. Single colony picks were examined on congo red-agar[5], pesticin 1 agar[6], and magnesium oxalate agar[7]. A clone demonstrating pigmentation, pesticin 1 and calcium dependence, all indicators of virulence, was chosen for inoculation into laboratory mice (ICR strain) and rats (Wistar strain)[8]. This clone, strain CPS-2a, was cultured at 25 °C in 2% peptone broth, diluted in the same medium, and inoculated s.c. into animals. Data on the virulence of strain CPS-2a were compared to data for the F1− strain CPS-1, studied previously[3], and for the F1+ *Y.pestis* strain 195/P.

In another experiment, carcasses of mice that had died from infection with F1− *Y.pestis* strain CPS-2a were fed to 20 rats. After several days without food, each rat was given 1 mouse carcass. Rats were moved into clean cages 36 h later and fed their normal laboratory chow. Rats that died were necropsied. Isolation from the spleen, on blood agar, was attempted to prove plague infection. Bacteremia was confirmed by isolation of bacilli from blood.

Results. Strain CPS-2a was of considerably greater virulence for laboratory rodents than the F1− *Y.pestis* strain CPS-1 (table 1). Strain CPS-2a was almost as lethal for mice as the typical F1+ *Y.pestis* strain 195/P. However, inoculation of moderate infective doses of strain CPS-2a killed only 2 rats (20%). F1− *Y.pestis* were isolated from the spleens of both. All rats fed carcasses of infected mice appeared sick 4–5 days later, and

Table 1. Virulence of F1− *Yersinia pestis* for laboratory rodents

	F1− *Y.pestis* strain CPS-1	F1− *Y.pestis* strain CPS-2a	F1+ *Y.pestis* strain 195/P
No. bacilli per mouse LD_{50}, 2 weeks post inoculation	> 502,000	60	35
No. bacilli inoculated into rats	50,000–600,000	190–1,900	1,450
No. rats dead/no. inoculated, 2 weeks post inoculation	0/8	2/10	10/10