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Gender-related differences in murine T- and B-lymphocyte proliferative ability in response to in vivo [Met^5]enkephalin administration

Jelka Gabrilovac *, Tanja Marotti

Division of Molecular Medicine, Rudjer Bošković Institute, Bijenička c. 54, PO Box 1016, HR-10000 Zagreb, Croatia

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Abstract

Gender-related differences in response to drugs of abuse, such as cocaine and morphine, have been reported both in humans and in experimental animals. Besides causing analgesia, morphine has recently been shown to exert strong immunosuppressive activity. However, no data on the influence of gender on the immunomodulatory effects of morphine or opioid peptides have been reported yet. The aim of this study was to test the influence of gender on the immunomodulatory ability of the endogenous opioid peptide [Met^5]enkephalin (MENK) in mice. This was done by comparing the proliferative ability of splenic T- and B-lymphocytes 14 h after systemic (intraperitoneal; i.p.) administration of [Met^5]enkephalin (2.5, 5 or 10 mg/kg body weight). The proliferative ability of T- and B-lymphocytes was assessed by testing their in vitro response to graded concentrations of the T- and B-cell mitogens, concanavalin-A (Con-A) and lipopolysaccharide (LPS), respectively. The results obtained showed that [Met^5]enkephalin, at a dose of 2.5 mg/kg, enhanced the proliferative ability of T-lymphocytes in male mice, but not in female mice. Similarly, [Met^5]enkephalin, at doses of 2.5 and 5 mg/kg, enhanced the proliferative ability of splenic B-lymphocytes in male mice, whereas in female mice a decrease was observed ([Met^5]enkephalin 2.5 mg/kg, LPS 10 μg/ml). [Met^5]enkephalin, at a dose of 10 mg/kg, did not affect the proliferative ability of either lymphocyte population, regardless of gender. The [Met^5]enkephalin-induced stimulatory effect on both T- and B-lymphocyte proliferation was reversed by naloxone (10 mg/kg body weight), injected prior to [Met^5]enkephalin, suggesting an involvement of opioid receptors. Thus, the data presented provide evidence for the gender-related response of murine splenic lymphocytes to immunomodulation by [Met^5]enkephalin, administered in vivo. This finding may be relevant to the potential use of [Met^5]enkephalin in adjuvant therapy for immunocompromised states, such as acquired immunodeficiency syndrome (AIDS) or malignancies.

Keywords: [Met^5]enkephalin; Gender; T-lymphocyte; B-lymphocyte; Proliferation; Opioid receptor

1. Introduction

[Leu^5]- and [Met^5]enkephalin are endogenous opioid peptides that, in addition to their neurotransmitter function, have significant immunomodulatory ability. Enhancement of various immune functions has been predominantly reported following their administration in vivo. Augmented humoral and cellular immune responses (Radulović and Janković, 1994), phagocytosis (Marotti et al., 1996), natural killer-cell activity (Gabrilovac et al., 1992; Kowalski, 1997), interferon-γ secretion (Gabrilovac et al., 1996), and lymphocyte proliferation (Kowalski, 1998) were found in rats or mice injected with enkephalins. Occasionally, however, opposite results, showing a decrease of some immune functions by enkephalins, have been reported as well (Janković and Marić, 1988; Gabrilovac et al., 1992; Marotti et al., 1993). Due to their predominant immunostimulatory activity, enkephalins have been proposed as adjuvant therapy for immunocompromised states such as acquired immunodeficiency syndrome (AIDS) (Plotnikoff et al., 1986; Wybran et al., 1987) and malignancies (Faith and Murgo, 1988).

Enkephalins may affect the immune response both indirectly, by binding to opioid receptors in the brain (Radu-

* Corresponding author. Tel.: +385-1-45-61-082; fax: +385-1-46-80-094.

E-mail address: gabril@rudjer.irb.hr (J. Gabrilovac).

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lović and Janković, 1994), and/or directly, by binding to opioid receptors on immunocytes. Namely, immunocytes have recently been shown to express opioid receptors of the μ, δ and κ class, which are highly homologous to those in brain (reviewed by Sharp et al., 1998). In addition, immunocytes, upon stimulation, secrete endogenous opioids (Linner et al., 1991), which act as local regulatory factors in both an autocrine and a paracrine fashion. It should be noted that the effects of a direct action of enkephalins may be opposite to those of an indirect action via the central nervous system and subsequent activation of the noradrenergic pathway. Therefore, the final outcome of the response to enkephalins in vivo probably represents the sum of the indirect and direct mechanisms of their action.

In our previous work, we studied immunomodulation by [Met^5]enkephalin in mice of CBA strain and found that it is greatly influenced by immunisation (Marotti et al., 1993), adrenalectomy (Marotti et al., 1992), and stress (Marotti et al., 1996). Each of the treatments significantly affected the response to immunomodulation by [Met^5]enkephalin, either by enhancing or attenuating it, or even by changing the direction of the effect. These data collectively stressed the importance of adrenal steroids in the immunomodulation produced by [Met^5]enkephalin, and that the treatments applied are associated with alterations in glucocorticoid level.

In addition to the adrenal steroids, gonadal steroids may also play an important role in immunomodulation by opioids. Sexual dimorphism of the nervous system has been well documented (Breedlove, 1992). A major role in the anatomical and functional dimorphism of the brain has been ascribed to steroid hormones (Kawata et al., 1994). Gender-related differences in binding ability to opioid receptors in certain brain regions (hypothalamus and striatum) of rats have been reported by Rimanoczy and Vathy (1995). Gender-related differences in the amount of opioid peptide in the medial preoptic area of rats, at the level of mRNA for preproenkephalin, have also been reported (Segarra et al., 1998). Consequently, several studies have reported gender-related differences in the antinociceptive response to the non-peptide opioid, morphine (Kepler et al., 1991; Cicero et al., 1996). In addition to antinociception, morphine exerts strong immunosuppresive activity (Bryant and Roudebush, 1990). However, there is no data on the role of gender in the immunosuppressive effect of morphine. The immunomodulatory activity of cocaine, another psycho-active drug, which in its multiple mechanisms of action may involve opioid receptors as well, has been shown to be gender related (Matulka et al., 1996).

The purpose of this study was to assess the possible influence of gender on immunomodulation by [Met^5]enkephalin. This was done by comparing the proliferative ability of splenic T- and B-lymphocytes in male and female CBA mice 14 h after systemic intraperitoneal (i.p.) injection of [Met^5]enkephalin (2.5, 5 and 10 mg/kg body weight). Involvement of opioid receptors in the [Met^5]enkephalin-induced immunomodulation was assessed by pretreatment of mice with naloxone.

2. Materials and methods

2.1. Animals

Male and female mice of the CBA/H$_{Zgr}$ inbred strain, 8–12 weeks old, were obtained from our conventional mouse colony. They were kept four per cage at controlled room temperature and under standard light conditions. They received standard pelleted food and had free access to water.

2.2. Chemicals

[Met^5]enkephalin (Sigma, Cat. No. L-9133) and naloxone (Sigma, Cat. No. N-7758) were kept at −20°C and were dissolved in phosphate-buffered saline (PBS) immediately before use. Mitogens, concanavalin-A (Con-A, Type V, Sigma, Cat. No. C-7275) and lipopolysaccharide (LPS, Sigma Cat. No. L-4391) were dissolved in PBS and kept as stock solutions at −20°C until use. 3-(4,5-Dimetylthiazol-2-yl)-2,5-diphenyltetrasolium bromide (MTT, Sigma, Cat. No. M-2128) was dissolved in PBS and kept as a stock solution (5 mg/kg) at −20°C until use.

2.3. Preparation of spleen cell suspensions

Mouse spleens were removed aseptically, and individual cell suspensions were prepared under lamina flow. Briefly, spleen was minced and passed through a nylon sieve. After the erythrocytes were lysed with 0.82% NH_4Cl (10 min at 4°C), the debris was removed by passing the suspension through a sterile gauze. After centrifugation, the cells were resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco), L-glutamine (3 mM), HEPES (20 mM), penicillin (0.1 g/l), streptomycin (0.1 g/l). Cells were counted and adjusted to 5×10^6 per ml.

2.4. Proliferation assay

The colorimetric MTT assay, originally described by Mosmann (1983), was used. Briefly, spleen cells (5×10^5 in 100 μl) were mixed with Con-A (in 50 μl, final concentrations 10, 5, 2.5, 1.25 and 0.6 μg/ml) in 96-well flat-bottomed microtiter plates (Becton Dickinson) and incubated for 48 h at 37°C in a humidified atmosphere with 5% CO_2. One hour before the end of the incubation, MTT (in 15 μl, final concentration 0.5 mg/ml) was added and the incubation was continued for 1 h at 37°C. Subse-

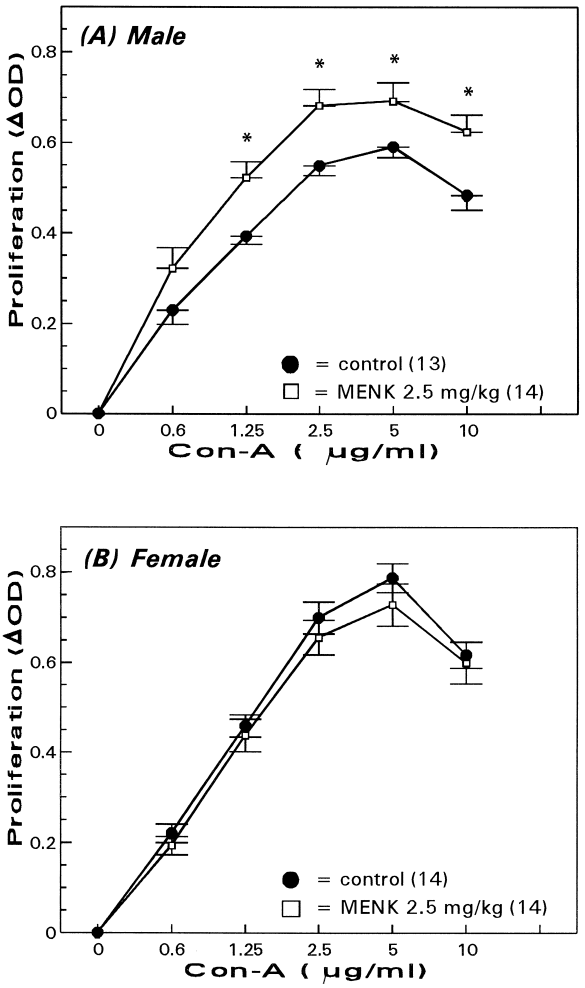

Fig. 1. Gender-related difference in T-lymphocyte proliferative ability in response to in vivo administered [Met^5]enkephalin. Male or female mice received one i.p. injection of [Met^5]enkephalin (MENK, 2.5 mg/kg). Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of T-cell mitogen, Con-A. Results are expressed as means ± S.E.M. obtained in four experiments. Numbers in brackets represent number of mice. $^*P <$ 0.05 (*t*-test).

quently, 100 μl of acidified isopropanol (0.04 N HCl isopropanol) was added to each well to dissolve the crystals of formazan. Non-dissolved crystals were mechanically disrupted by the multichannel pipettor. Optical density (OD) was determined on an ELISA reader at 570 nm wavelength. Each sample was run in quadruplicate. The precision of the MTT test (i.e. [S.D./mean] × 100) was up to 7%. The data are expressed as ΔOD at 570 nm. ΔOD was calculated as the difference between samples with and without mitogen.

2.5. Experimental design

Male and female mice of the experimental groups received an i.p. injection of [Met^5]enkephalin (2.5, 5 or 10 mg/kg body weight in 0.5 ml). Control mice received PBS. The animals were killed by cervical dislocation 14 h after the injection. The blocking effect of naloxone was tested by injecting naloxone (10 mg/kg body weight in 0.1 ml) 20 min before [Met^5]enkephalin. Control mice received naloxone followed by PBS. Individual suspensions of spleen cells were prepared and tested for proliferation in response to Con-A or LPS after 48 h of incubation with mitogen.

2.6. Statistics

The differences between the groups were checked by analysis of variance (ANOVA) followed by the *t*-test. The significance level was set at $P < 0.05$.

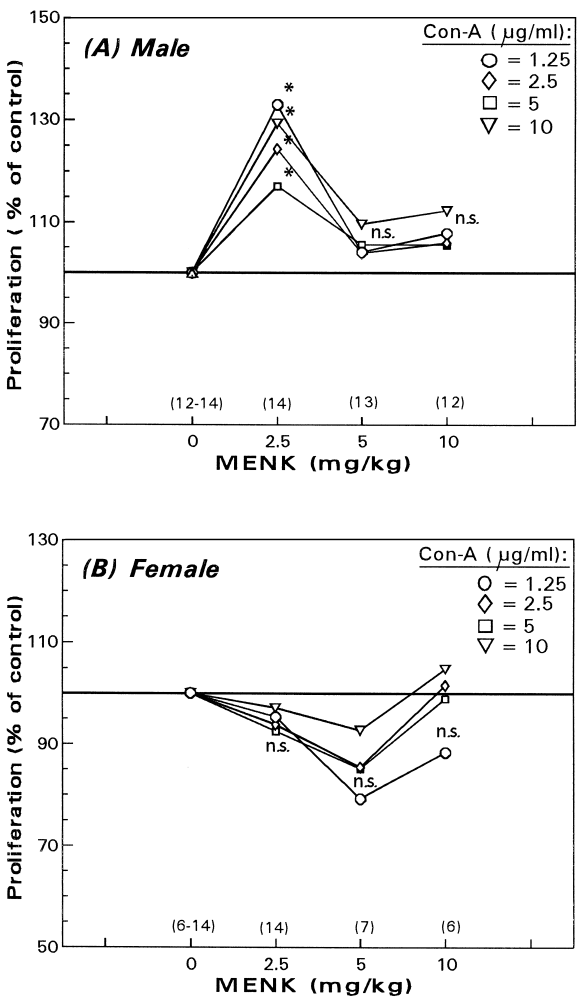

Fig. 2. Gender-related difference in T-lymphocyte proliferative ability in response to graded doses of in vivo administered [Met^5]enkephalin. Male or female mice received one i.p. injection of [Met^5]enkephalin (MENK) containing 2.5, 5 or 10 mg/kg. Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of Con-A. Results are expressed as percentages of the control (mice injected with PBS) and were obtained in 12 experiments. Numbers in brackets represent number of mice. $^*P < 0.05$ (*t*-test).

3. Results

3.1. Gender-related modulation of T-lymphocyte proliferative ability induced by in vivo treatment with [Met^5]enkephalin

In male mice one i.p. injection of [Met^5]enkephalin (2.5 mg/kg) enhanced the in vitro response of their splenic T-lymphocytes to Con-A (Fig. 1A; $F(1,133) = 12.91$; $P = 0.001$). A significant increase in proliferation ($P < 0.05$; *t*-test) was found with all Con-A concentrations, except for the lowest one (0.6 μg/ml). In contrast to male mice, in female mice injection of [Met^5]enkephalin (2.5 mg/kg) did not affect the Con-A response of their splenic T-lymphocytes (Fig. 1B; $F(1,138) = 0.739$; $P = 0.40$).

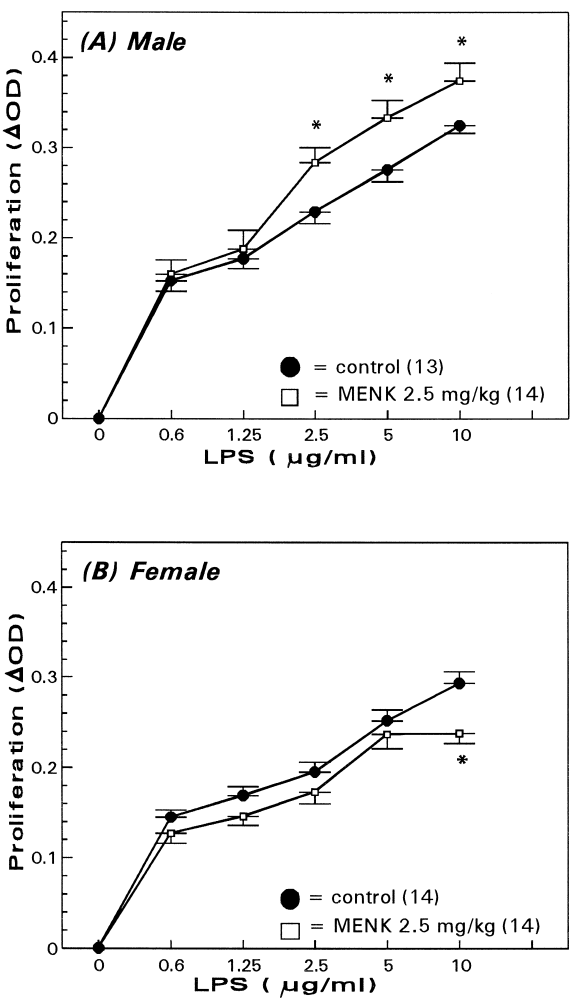

Fig. 3. Gender-related difference in B-lymphocyte proliferative ability in response to in vivo administered [Met^5]enkephalin. Male or female mice received one i.p. injection of [Met^5]enkephalin (MENK, 2.5 mg/kg). Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of B-cell mitogen, LPS. Results are expressed as means ± S.E.M. obtained in four experiments. Numbers in brackets represent number of mice. $^*P < 0.05$ (*t*-test).

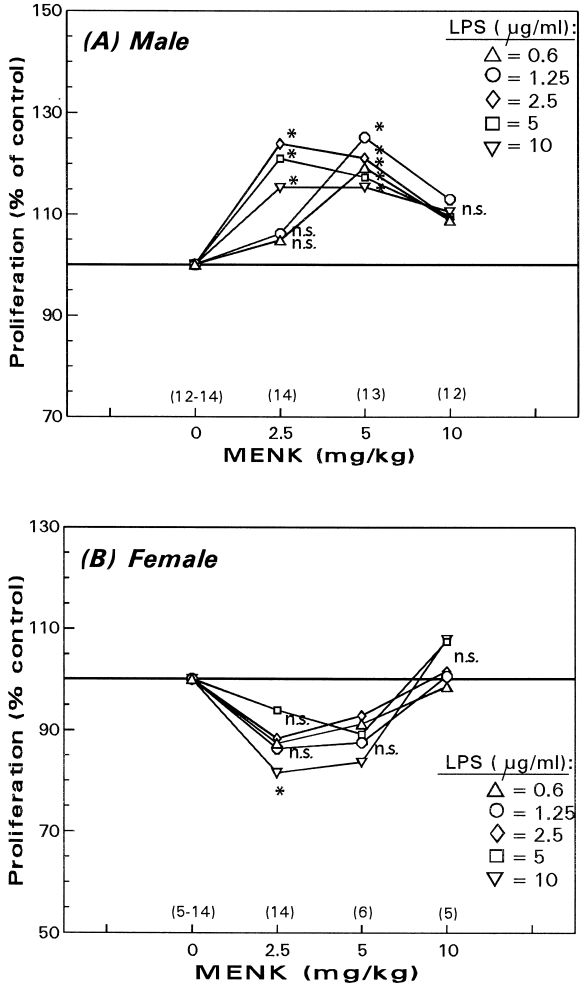

Fig. 4. Gender-related difference in B-lymphocyte proliferative ability in response to graded doses of in vivo administered [Met^5]enkephalin. Male or female mice received one i.p. injection of [Met^5]enkephalin (MENK) containing 2.5, 5 or 10 mg/kg. Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of B-cell mitogen, LPS. Results are expressed as percentage of the control (mice injected with PBS) and were obtained in 12 experiments. Numbers in brackets represent number of mice. $^*P < 0.05$ (*t*-test).

Higher doses of [Met^5]enkephalin (5 or 10 mg/kg) did not augment T-lymphocyte proliferation in male (for [Met^5]enkephalin 5 mg/kg: $F(1,133) = 0.753$; $P = 0.3965$; for [Met^5]enkephalin = 10 mg/kg : $F(1,123) = 1.131$; $P = 0.2896$) (Fig. 2A) or female mice (Fig. 2B). On the contrary, in female mice, [Met^5]enkephalin at a dose of 5 mg/kg even tended to decrease the Con-A response (Fig. 2B; $F(1,68) = 2.296$; $P = 0.1343$), whereas a dose of 10 mg/kg of [Met^5]enkephalin was ineffective (Fig. 2B; $F(1,58) = 0.058$; $P = 0.8109$).

3.2. Gender-related modulation of B-lymphocyte proliferative ability induced by in vivo treatment with [Met^5]enkephalin

In male mice one i.p. injection of [Met^5]enkephalin (2.5 mg/kg) significantly enhanced the in vitro response of

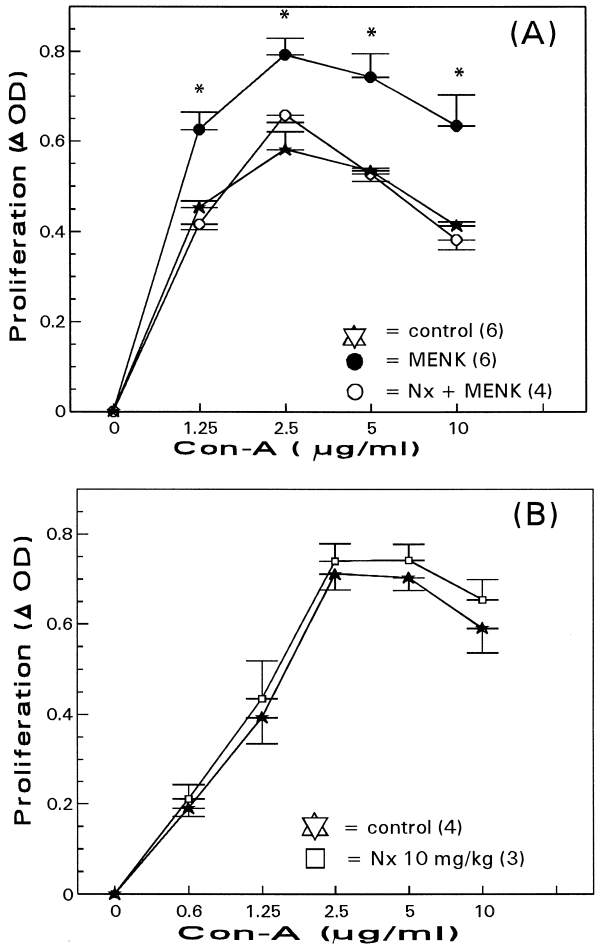

Fig. 5. Naloxone reverses the [Met^5]enkephalin-induced enhancement of T-lymphocyte proliferative ability. (A) Male mice received one i.p. injection of either: PBS (control), [Met^5]enkephalin (MENK, 2.5 mg/kg) or naloxone (Nx, 10 mg/kg) followed by [Met^5]enkephalin. (B) Male mice received one i.p. injection of either PBS (control), or naloxone (Nx, 10 mg/kg). Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of T-cell mitogen, Con-A. Results are expressed as means ± S.E.M. and were obtained in two (A) or one (B) experiment(s). Numbers in brackets represent number of mice. * [Met^5]enkephalin (MENK) significantly ($P < 0.05$) different from the control as well as from naloxon + [Met^5]enkephalin (Nx + MENK).

their splenic B-lymphocytes to LPS (Fig. 3A; $F(1,133) = 5.059$; $P = 0.026$). This was also true for higher LPS concentrations (for LPS 10, 5 and 2.5 μg/ml, $P < 0.05$; *t*-test). Injection of [Met^5]enkephalin at the same dose (2.5 mg/kg) into female CBA mice, in contrast, attenuated the LPS activation (Fig. 3B; $F(1,138) = 5.736$; $P = 0.0180$). This was true for LPS at the highest concentration (10 μg/ml; $P = 0.005$). A higher dose of [Met^5]enkephalin (5 mg/kg) significantly enhanced the LPS response in male mice (Fig. 4A; $F(1,133) = 6.536$; $P = 0.0117$), but in female mice tended to suppress the LPS response of their splenic B-lymphocytes (Fig. 4B; $F(1,58) = 2.214$; $P = 0.1422$). [Met^5]enkephalin at a concentration of 10 mg/kg was ineffective both in male and female mice (Fig. 4A and B) (for male mice: $F(1,123) = 1.709$; $P = 0.1935$; for female mice: $F(1,48) = 0.208$; $P = 0.625$).

3.3. [Met^5]enkephalin-induced enhancement of T-lymphocyte proliferative ability is reversed by naloxone

In order to assess the role of opioid receptors in [Met^5]enkephalin-induced enhancement of Con-A-induced

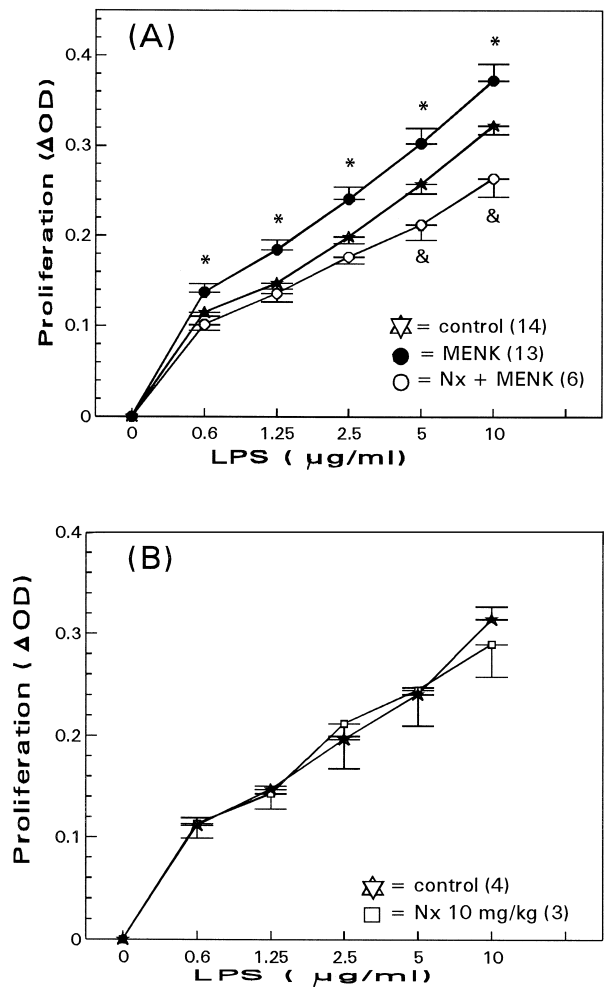

Fig. 6. Naloxone reverses the [Met^5]enkephalin-induced enhancement of B-lymphocyte proliferative ability. (A) Male mice received one i.p. injection of either: PBS (control), [Met^5]enkephalin (MENK, 5 mg/kg) or naloxone (Nx, 10 mg/kg) followed by [Met^5]enkephalin. (B) Male mice received one i.p. injection of either PBS (control) or naloxone (Nx, 10 mg/kg). Fourteen hours later their spleens were removed and individually tested for proliferative ability in the presence of graded concentrations of B-cell mitogen, LPS. Results are expressed as means ± S.E.M. and were obtained in three (A) or one (B) experiment(s). Numbers in brackets represent number of mice. * [Met^5]enkephalin (MENK) significantly ($P < 0.05$) different from the control as well as from naloxone + [Met^5]enkephalin (Nx + MENK); and Nx + MENK significantly ($P < 0.05$) different from the control.

T-lymphocyte proliferation in male mice, naloxone (10 mg/kg) was injected 20 min prior to [Met^5]enkephalin (2.5 mg/kg). Again, [Met^5]enkephalin enhanced T-lymphocyte proliferation ($F(1,59) = 35.289$; $P = 0.0001$) (Fig. 5A). Naloxone reversed the enhancing effect of [Met^5]enkephalin on proliferation induced by Con-A ($F(1,49) = 16.792$; $P = 0.0002$) (Fig. 5A), whereas naloxone itself had no effect on Con-A-induced T-cell proliferation ($F(1,34) = 0.25$; $P = 0.609$) (Fig. 5B).

3.4. [Met^5]enkephalin-induced enhancement of B-lymphocyte proliferative ability is reversed by naloxone

Similarly, the role of opioid receptors in the [Met^5]enkephalin-induced enhancement of LPS-induced B-lymphocyte proliferation in male mice was tested by injecting naloxone (10 mg/kg) 20 min prior to [Met^5]enkephalin (5 mg/kg). [Met^5]enkephalin at a dose of 5 mg/kg enhanced B-lymphocyte proliferation (Fig. 6; $F(1,133) = 6.536$; $P = 0.011$). This was true for all LPS concentrations tested ($P < 0.05$, *t*-test). The [Met^5]enkephalin-induced enhancement of the LPS response was fully reversed by naloxone (Fig. 6; $F(1,98) = 3.321$; $P = 0.07$). At higher LPS concentrations naloxone decreased the LPS response to below the control level (Fig. 6A) (for LPS 10 and 5 mg/kg: $P = 0.01$ and 0.03, respectively). Naloxone itself had no effect on B cell proliferation (Fig. 6B; $F(1,33) = 0.04$; $P = 0.9491$).

4. Discussion

The data presented in this paper suggest a gender-related difference in the response of murine T- and B-lymphocytes to in vivo administration of the endogenous opioid [Met^5]enkephalin, as assayed by an altered proliferative ability in vitro. [Met^5]enkephalin at a dose of 2.5 mg/kg enhanced both T- and B-cell proliferation in male mice, but had no effect on T-cell proliferation and even attenuated B-cell proliferation in female mice. At a dose of 5 mg/kg, [Met^5]enkephalin enhanced B-cell proliferation in male mice and tended to decrease it in female mice. [Met^5]enkephalin at a dose of 10 mg/kg did not alter either lymphocyte population, regardless of the gender of the mice. Involvement of opioid receptors in the observed stimulatory effect of [Met^5]enkephalin on T- and B-lymphocyte proliferation in male mice was confirmed by its complete reversal with the opioid receptor antagonist naloxone.

Thus, the data of this paper provide evidence for a gender-related difference in the response of mouse lymphocytes to the endogenous opioid peptide [Met^5]enkephalin. To our knowledge, this is the first report studying the role of gender on the effect of in vivo administered endogenous opioid peptide on lymphocyte function. In vitro studies of Brummit et al. (1988) have shown that β-endorphin decreases the secretion of interferon-γ by peripheral blood mononuclear cells from male, but not female, donors. Recently, Matulka et al. (1996) reported gender-related differences in the immunomodulatory (stimulation with low, and suppression with high concentrations) response of rats to cocaine. Again, males were found to be more sensitive, as the effects were obtained with lower concentrations of cocaine in male than in female rats. The lack of a stimulatory effect of [Met^5]enkephalin in female mice observed in this study may be due to the relatively narrow range of [Met^5]enkephalin concentrations tested: lower or higher [Met^5]enkephalin concentrations may have elicited a response in lymphocytes from female mice. Recently, Kowalski (1998) reported a stimulatory effect of low concentrations of [Met^5]enkephalin on lymphocytes from female C57Bl mice. Concentrations as low as 50 μg/kg body weight, injected systemically (i.p.), were effective in enhancing the in vitro proliferation of T- and B-splenic lymphocytes.

The immunostimulatory ability of [Met^5]enkephalin observed in male mice exhibited a bell-shaped dose–response curve: T-lymphocyte proliferation was enhanced by a dose of 2.5 mg/kg, and B-lymphocytes by doses of 2.5 and 5 mg/kg, whereas a dose of 10 mg/kg of [Met^5]enkephalin was ineffective in modulating the proliferation of either lymphocyte population. The bell-shaped dose–response curve of [Met^5]enkephalin in enhancing T-lymphocyte proliferation in male mice observed in this study resembles that obtained by Radulović and Janković (1994) after intracerebral administration of [Met^5]enkephalin into male rats. A dose of 1 μg/kg body weight was shown to be the most effective in enhancing the number of antibody-forming cells, whereas higher doses exerted no effect. The bell-shaped dose–response curve of [Met^5] enkephalin suggests the involvement of other regulatory mechanism(s) at higher doses.

In our previous studies with [Met^5]enkephalin we used male CBA mice (Marotti et al., 1996). Injected at a dose of 10 mg/kg body weight, [Met^5]enkephalin exerted no effect on Con-A-driven T-lymphocyte proliferation. However, this dose of [Met^5]enkephalin fully reversed the inhibitory effect of stress (Marotti et al., 1996), suggesting that both the effect of an opioid peptide and its effective dose are complexly regulated and may depend on many parameters.

Gender has been reported to affect the influence of non-peptide opioids with μ-opioid receptor specificity, such as morphine, on the modulation of antinociception (Kepler et al., 1989; Islam et al., 1993), but there are no such reports for endogenous opioid peptides. Generally, male rats were more susceptible than female rats, as assessed by several antinociceptive tests, such as tail-flick, hot-plate and abdominal-constriction tests (Cicero et al., 1996). Thus, male animals seem to be more susceptible to

both peptide and non-peptide opioid effects on immune and antinociceptive responses, respectively.

The gender-related difference in the response of murine lymphocytes to [Met^5]enkephalin, found in this study, may be due to differences in: (a) sex-steroid hormones; (b) opioid receptor expression and/or sensitivity; and (c) enzymes which degrade [Met^5]enkephalin. (a) The role of sex-steroid hormones in the gender-related response to morphine was proposed by Kasson et al. (1983), but variable results have been obtained: Kepler et al. (1989) and Cicero et al. (1996) reported no effect of castration, whereas Islam et al. (1993) observed an equalising effect of gonadectomy on sensitivity to morphine in males and females. No studies examining the influence of castration on immunomodulation by endogenous or exogenous opioids have been reported. (b) Sex-related dimorphism of the nervous system is well documented (Breedlove, 1992) and may imply that there are differences in opioid receptor expression and/or sensitivity. Indeed, Rimanoczy and Vanthy (1995) reported gender-related differences in binding capacity (B_{max}) to μ-opioid receptors in rat brain, the capacity being higher in male rats than in female rats. The gender-related binding ability was ascribed to steroids, as estrogens restored the binding ability in ovariectomised rats. The gender-related expression of opioid receptors found on neuronal cells might also apply to immunocytes, but we have found no such data in the literature yet. Up-regulation of δ-opioid receptors on T lymphocytes after stimulation with mitogen has recently been reported by Miller (1996). (c) Endogenous opioid peptides are readily degraded in vivo by means of soluble (Kerr and Kenny, 1974; Hambrook et al., 1976) and membrane-bound aminopeptidases and carboxipeptidases (Roscetti et al., 1990). The activity of some of these enzymes was shown to be significantly higher in female than in male donors (Schweisfurth et al., 1984; Kashimata et al., 1985; Martinez et al., 1998). Thus, the lack of a response to [Met^5]enkephalin in female mice observed in this study might reflect the more rapid degradation of [Met^5]enkephalin in female vs. male mice.

Collectively, the greater susceptibility of males to [Met^5]enkephalin immunomodulation may reflect a superior binding activity to opioid receptors and a longer availability of the peptide due to relatively lower levels/activity of enkephalin-degrading enzymes.

In conclusion, this study has shown gender-related differences in murine T- and B-lymphocyte proliferative ability in response to in vivo treatment with the endogenous opioid peptide [Met^5]enkephalin. A stimulatory effect of [Met^5]enkephalin on the proliferative ability of T- and B-cells was found in male mice, but not in female mice. The enhancement of T- and B-cell proliferative ability by [Met^5]enkephalin was opioid-receptor mediated, as it could be reversed by naloxone. The observed gender-related response to [Met^5]enkephalin is in agreement with several reports showing a higher susceptibility of males to morphine, and may be relevant to the potential use of [Met^5]enkephalin as an immunostimulator in adjuvant therapy of AIDS or malignancies.

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References

Breedlove, S.C., 1992. Sexual dimorphism in the vertebrate nervous system. J. Neurosci. 12, 4133–4142.

Brummitt, C.F., Sharp, B.M., Gekker, G., Keane, W.F., Peterson, P.K., 1988. Modulatory effects of β-endorphin on interferon-γ production by cultured peripheral blood mononuclear cells: heterogeneity among donors and the influence of culture medium. Brain Behav. Immunol. 2, 187–197.

Bryant, H.U., Roudebush, R.E., 1990. Suppressive effects of morphine pellet implants on in vivo parameters of immune function. J. Pharmacol. Exp. Ther. 255, 410–414.

Cicero, T.J., Nock, B., Meyer, E.R., 1996. Gender-related differences in the antinociceptive properties of morphine. J. Pharmacol. Exp. Ther. 279, 767–773.

Faith, R.E., Murgo, A.J., 1988. Inhibition of pulmonary metastases and enhancement of natural killer cell activity by methionine–enkephalin. Brain Behav. Immunol. 2, 114–122.

Gabrilovac, J., Antica, M., Osmak, M., 1992. In vivo bidirectional modulation of mouse natural killer (NK) cell cytotoxic activities by leu-enkephalin. Life Sci. 50, 29–37.

Gabrilovac, J., Ikić-Sutlić, M., Knežević, N., Poljak, Lj., 1996. Leu-enkephalin enhances interferon secretion in mice. Res. Exp. Med. 196, 137–144.

Hambrook, J.M., Morgan, B.A., Rance, M.J., Smith, C.F., 1976. Mode of deactivation of the enkephalins by rat of human plasma and rat brain homogenates. Nature 262, 782–783.

Islam, A.K., Cooper, M.L., Bodnar, R.J., 1993. Interaction among ageing, gender and gonadectomy effects upon morphine antinociception in rats. Physiol. Behav. 54, 45–53.

Janković, B.D., Marić, D., 1988. Enkephalins modulate in vivo immune reactions through delta- and mu-opioid receptors. Ann. NY Acad. Sci. 540, 691–693.

Kashimata, M., Hiramatsu, M., Kumegawa, M., Minami, N., 1985. Sexual differences in kininase activity in the mouse submandibular gland. Enzyme 34, 22–26.

Kasson, B.G., Carlson, H.E., George, R., 1983. Endocrine influences on the actions of morphine: II. Responses to pituitary hormones. J. Pharmacol. Exp. Ther. 224, 282–288.

Kawata, M., Yuri, K., Morimoto, M., 1994. Steroid hormone effects on gene expression, neuronal structure, and differentiation. Horm. Behav. 28, 477–482.

Kepler, K.L., Kest, B., Kiefel, J.M., Cooper, M.L., Bodnar, R.J., 1989. Role of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats. Pharmacol. Biochem. Behav. 34, 119–127.

Kepler, K.L., Standifer, K.M., Paul, D., Kest, B., Pasternak, G.W., Bodnar, R.J., 1991. Gender effects and central opioid analgesia. Pain 45, 87–94.

Kerr, M.A., Kenny, A.J., 1974. The purification and specificity of a

neutral endopeptidase from rabbit kidney brush border. Biochem. J. 137, 477–488.

Kowalski, J., 1997. Effect of enkephalins and endorphins on cytotoxic activity of natural killer cells and macrophages/monocytes in mice. Eur. J. Pharmacol. 326, 251–255.

Kowalski, J., 1998. Immunologic action of [Met^5]enkephalin fragments. Eur. J. Pharmacol. 347, 95–99.

Linner, K.M., Beyer, H.S., Sharp, B.M., 1991. Induction of the messenger ribonucleic acid for proenkephalin A in cultured murine CD4-positive thymocytes. Endocrinology 128, 717–724.

Marotti, T., Gabrilovac, J., Rabatić, S., Šmejkal-Jagar, L., Ročić, B., Haberstock, H., 1996. Met-enkephalin modulates stress-induced alterations of the immune response in mice. Pharmacol. Biochem. Behav. 54, 277–284.

Marotti, T., Rabatić, S., Gabrilovac, J., 1993. A characterization of in vivo immunomodulation by Met-enkephalin in mice. Int. J. Imunopharmacol. 15, 919–926.

Marotti, T., Ročić, B., Gabrilovac, J., Haberstock, H., 1992. Interaction of Met-enkephalin and corticosteroids in immunomodulation. Int. J. Immunopharmacol. 14, 621–627.

Martinez, J.M., Ramirrez, M.J., Prieto, I., Alba, F., Ramirez, M., 1998. Sex differences and in vitro effects of steroids on serum aminopeptidase activities. Peptides 19, 1637–1640.

Matulka, R.A., Jordan, S.D., Stanulis, E.D., Holsapple, M.P., 1996. Evaluation of strain and sex-dependency of cocaine-induced immunosuppression in B6C3F1 and DBA/2 mice. J. Pharmacol. Exp. Ther. 279, 12–17.

Miller, B., 1996. δ opioid receptor expression is induced by concanavalin A in $CD4^+$ T cells. J. Immunol. 157, 5324–5328.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Plotnikoff, N.P., Wybran, J., Nimeh, N.F., Miller, G.C., 1986. Methionine-enkephalin: enhancement of T-cells in patients with Kaposi sarcoma (AIDS). Psychopharmacol. Bull. 22, 695–697.

Radulović, J., Janković, B., 1994. Opposing activities of brain opioid receptors in the regulation of humoral and cell-mediated immune response in the rat. Brain Res. 661, 189–195.

Rimanoczy, A., Vathy, I., 1995. Prenatal exposure to morphine alters brain μ opioid receptor characteristics. Brain Res. 690, 245–248.

Roscetti, G., Bongiorno, L., Urbani, A., Marini, M., Roda, L.G., 1990. Hydrolysis and binding of leucine-enkephalin to lymphomic and erythroleukaemic cell lines. Int. J. Immunopharmacol. 12, 391–396.

Schweisfurth, H., Heinrich, J., Brugger, E., Burghardt, W., 1984. Changes in serum carboxipeptidase N in patients with lung sarcoidosis. Deutsche Med. Wochenschr. 109, 166–169.

Segarra, A.C., Acosta, A.M., Gozales, J.L., Angulo, J.A., McEwen, B.S., 1998. Sex differences in estrogenic regulation of preproenkephalin mRNA levels in the medial preoptic area of prepubertal rats. Mol. Brain Res. 60, 133–139.

Sharp, B.M., Roy, S., Bidlack, J.M., 1998. Evidence for opioid receptors on cells involved in host defence and the immune system. J. Neuroimmunol. 83, 45–56.

Wybran, J., Schandene, L., VanVooren, J.P., Vandermoten, G., Latinne, D., Sonnet, J., de Bruyere, M., Taelman, H., Plotnikoff, N.P., 1987. Immunological properties of methionine-enkephalin, and therapeutic implications in AIDS, ARC, and cancer. Ann. NY Acad. Sci. 496, 108–114.