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Met-Enkephalin Modulates Stress-Induced Alterations of the Immune Response in Mice

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MAROTTI, T., J. GABRILOVAC, S. RABATIC, L. SMEJKAL-JAGAR, B. ROCIC AND H. HABERSTOCK. *Met-enkephalin modulates stress-induced alterations of the immune response in mice.* PHARMACOL BIOCHEM BEHAV **54**(1) 277–284, 1996.—Overnight restraint stress of mice decreased ConA-driven lymphocyte proliferation, plaque-forming cell response to sheep red blood cells (SRBC), and NK activity in the spleen, but the phagocytic activity was enhanced. Injection of methionine-enkephalin (MENK), 10 mg/kg, IP, 30 min before restraint, abolished these changes (except for the NK activity) and attenuated the stress-induced elevation of glucocorticoids. However, MENK itself affected the immune responses like stress: It decreased NK activity and the PFC response and enhanced phagocytic activity. Contrary to results with stress, MENK had no effect on cell proliferation. The opioid-receptor antagonist naloxone given before restraint reversed the stress-induced enhancement of phagocytosis and the decrease of T-cell proliferation. Alterations of the immune responses induced by restraint stress seem to be mediated by at least two mechanisms: activation of the hypothalamus–pituitary–adrenal (HPA) axis and the secretion of opioid peptides. MENK injected before stress may interfere with either or both mechanisms. T or B lymphocytes seem to be affected by the activation of the HPA axis, and phagocytes by a direct opioid action, whereas NK cells seem to be under the influence of another control mechanism.

Met-enkephalin Stress Immune response Naloxone HPA axis

WE HAVE previously shown that single and multiple intraperitoneal (IP) injection(s) of the endogenous opioid peptide Met-enkephalin (MENK) modulated the immune response of mice (30,31). A single injection of MENK (10 mg/kg) suppressed, after 12 h, ConA-driven spleen cell proliferation, plaque-forming response to sheep red blood cells (SRBC), and the NK activity, as well as the phagocytic activity of peritoneal macrophages. Concomitantly with the suppression of immune functions, the plasma levels of corticotropin (ACTH) and corticosterone (CS) were elevated, suggesting a causal relationship with MENK injection. The immunosuppressive effects induced by MENK could be abrogated by adrenalectomy (ADx), as if an activation of the hypothalamus–pituitary–adrenal (HPA) axis mediated the inhibitory action of MENK (30). However, the MENK-induced alteration of NK cytotoxicity was resistant to ADx, indicating the involvement of other non-HPA mechanism(s) in MENK action. A direct interference with the immunocytes is possible, as T and B lymphocytes in mouse spleen express μ-, δ- (35), and κ-opioid receptors (4). The receptors have also been found on polymorphonuclear cells (15) and macrophages (7). The effects of binding of endogenous opioid peptides (42) or of synthetic selective agonists (43) have been studied in several in vitro models. MENK enhanced the proliferation of T-lymphocytes from human peripheral blood (22) and mouse spleen (33) NK cytotoxicity of human peripheral blood lymphocytes (PBL) (13) the phagocytic activity of rat macrophages and the secretion of chemotactic factor by human peripheral blood mononuclear cells (45). In our hands, the exposure of mouse peritoneal cells to MENK in vitro enhanced LPS-induced interleukin 1 (IL-1) secretion (29).

Emotional and/or physical stress is usually accompanied

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by a suppression of immune reactivity (10), which is attributed to an activation of the HPA axis resulting in elevated glucocorticoids, and to an increased noradrenergic output in the peripheral lymphoid organs (9). The condition of stress may be accompanied by peripheral analgesia caused by a secretion of opioid peptides (40). Some immunosuppressive effects of stress (e.g., decreased NK activity in the spleen) have been ascribed to endogenous opioids (40), as they could be abrogated by the opioid receptor antagonist naltrexone. Thus, MENK and stress both operate via activation of the HPA axis, and opioids may be released during stress (37).

The aim of this study was to examine the ability of the endogenous opioid pentapeptide MENK to interfere with stress-induced alterations of immune functions in mice. The animals received an IP injection of MENK before overnight restraint stress. ConA-driven proliferation, plaque-forming cell (PFC) response to SRBC, NK cytotoxicity, and the phagocytic activity of spleen cells harvested immediately after the cessation of stress were tested in vitro. Alterations in plasma CS levels were recorded simultaneously. By using the opioid antagonist naloxone, we examined whether MENK modulated the effects of stress by interfering with endogenously secreted opioid peptides.

METHOD

Animals

Male CBA/HZgr mice, 8–10 weeks old, produced in our mouse colony under SPF conditions, were used as donors of spleen cells and plasma. The animals were maintained in groups of three per cage, with food and water ad lib. The treatment groups included controls treated with saline (C); MENK treated (M); stressed (S); stressed and MENK treated (M + S); naloxone treated (NX); and stressed and naloxone treated (NX + S).

Drug Administration

One IP MENK (Sigma, St. Louis, MO) injection [10 mg/kg body wt. in Hank's balanced salt solution (HBSS)] proceeded for 30 min at the onset of the restraint stress (2000 h). That dose of MENK affects immune functions (23,34), inhibits the growth of human cancer (46) and tumour metastasis in mice (14), and prolongs the survival of mice with leukemia (34). It raises the plasma level of the enkephalin 10^4 times the physiologic level, although the enkephalin-degrading enzymes in the blood degrade that pentapeptide very rapidly (36). In some experiments, the animals were injected with NX (Sigma, 10 mg/kg) instead of MENK. Control animals received saline at the same time and in the same schedule. After restraint, the animals were anaesthetized with ether and bled from the jugular vein. Spleens were removed aseptically, minced, washed several times with HBSS, and counted using Trypan blue dye exclusion in a haemocytometer. Unfractioned spleen cells for all assays described were obtained from a single animal.

Stress Protocol

The mice were allowed to enter translucent restraint boxes (inner size 9 × 3 × 2.4 cm) preventing forward and backward movement but permitting free breathing. The restraint stress session lasted overnight (from 2000 to 0800 h). Control mice were kept in the home cages.

Proliferative Response to ConA

We added 100 μl of the splenocyte suspension (5×10^5 cells) in RPMI 1640 with 5% fetal calf serum (FCS) and 5×10^{-5} M 2-mercaptoethanol to the wells of flat-bottom 96-well plates (Falcon, NJ), together with 0.5 μg ConA per well in 100 μl of RPMI 1640. After incubation for 48 h in 5% CO_2 in humified air at 37°C, 1 μCi of [^{3}H]thymidine ([^{3}H]-TdR) (Amersham, Buckinghamshire, UK) with a spec. act. of 2 Ci/mmol) was added to each well. The cells were collected and washed 18 h later on glass fibre filters using the MASH II harvester (Microbiological Association, Bethesda, MD). The filters were dried at room temperature and the radioactivity was determined.

PFC Assay

We used a microplate method for in vitro immunization (32). Spleen cells were suspended in Iscove's modified Dulbecco's medium (Sigma) containing 8% heat-inactivated newborn calf serum (Rudjer Boskovic Institute), L-glutamine (2×10^{-6} M final concentration; Sigma), 2-mercaptoethanol (2×10^{-5} M final concentration; Sigma), penicillin (50 mg/l), and streptomycin (60 mg/l) (Pliva, Croatia). The spleen cell suspension was adjusted to 6×10^6/ml. Aliquots of 0.1 ml of cells were cultured in flat-bottom 96-well plates (Golijas, Slovenia) and immunized with 0.1 ml (10^7/ml) of washed SRBC (Imunoloski Zavod, Croatia). The cultures were incubated for 4 days in 5% CO_2 at 37°C, 100% humidity, on a rocking platform (10° inclination, 12 cycles/min). The PFC were assayed by Jerne's technique as modified in our laboratory (21). The content of each well was stirred by gentle pipeting, and 0.05 ml of the suspension was mixed with 0.5 ml of warm 0.5% agarose (IBF, France) solution in HBSS containing 1.5% SRBC. The mixture was poured over microscopic slides precoated with 1% agarose in phosphate-buffered saline (PBS). The slides were incubated for 1 h at 37°C in a humid atmosphere, and for an additional 2 h with 1 : 10 complement solution (Imunoloski Zavod). Visible plaques were scored and the PFC count was expressed per 10^6 viable cells.

Phagocytosis Assay

Unfractioned spleen cells (10×10^6/ml) were washed in Medium 199. Their ability to ingest opsonized ^{51}Cr-labelled SRBC was measured as following (38): ^{51}Cr-labelled SRBC (3.7 MBq/5 × 10^8 SRBC; spec. act. 7.4–18.5 GBq/mg; Amersham) opsonized with rabbit anti-SRBC serum were mixed with the effector cells (1 : 1) in a total volume of 300 μl Medium 199 containing 8 mM L-glutamine (Gibco, Grand Island, NY), penicillin (100 U/ml)-streptomycin (100 μg/ml) (Gibco), 10 mM Hepes buffer, and 10% FCS. The mixture was incubated at 37°C for 90 min. Phagocytosis was then stopped by placing the cells on ice. Nonphagocytosed SRBC were lysed by hypotonic medium in half of the samples. Lysed and nonlysed samples were centrifuged, the supernatants were collected and radioactivity was measured separately in the supernatants and in the pellets by a γ-counter. The phagocytosis was expressed as the radioactivity in the pellet, as the percentage of total radioactivity (in the pellet and in the supernatant).

NK Activity Assay

After removal of the erythrocytes by means of 0.84% NH_4Cl, the spleen cells (effectors) were resuspended in RPMI 1640 supplemented with 10% FCS and adjusted to 40×10^6, 20×10^6, and 10×10^6 cells/ml. Cells of the YAC-1 cell line served as targets. $3–5 \times 10^6$ YAC-1 cells were labelled by incubation with 200–300 μCi ^{51}Cr (Na_2CrO_4; Amersham) for 1 h at 37°C. After washing, the cells were resuspended in

RPMI 1640 with 10% FCS, and their concentration was adjusted to 2×10^5/ml; a 0.1-ml sample of the labelled target cell suspension was mixed with 0.1 ml effector cell suspension in appropriate concentrations, as to obtain the effector-to-target cell (E : T) ratios of 200, 100, and 50, and incubated for 4 h at 37°C in a humidified atmosphere with 5% CO_2.

All samples were done in triplicate and the mean percentage specific target cell lysis was calculated according to the equation: Specific target cell lysis (%) = $(E - C)/(M - C)$, where E is the percentage of lysis in samples containing effector and target cells, C the percentage of lysis in samples containing target cells only, and M the percentage of lysis in samples containing target cells and Triton X-100. Nonspecific chromium release (C) was between 7 and 16%. Maximal ^{51}Cr release obtained in the presence of Triton X-100 (M) was between 90 and 100%. Data are expressed as lytic unit (LU) per 10^7 spleen cells. One LU is defined as the number of cells causing 30% specific target cell lysis.

CS Radioimmunoassay (RIA)

Mice were bled into chilled polyethylene tubes containing 50 μl heparin (250 IU) (Rathiopharm, Germany) and immediately centrifuged at 4°C. The plasma obtained was transferred to polyethylene tubes containing 1000 IU trasylol (Bayer, Germany), frozen, and stored at −20°C until assay. CS concentration was determined in 50 μl of plasma by RIA using the [^{125}I]CS assay kit (ICN Biomedicals, Cosa Mesa, CA). The limit of sensitivity of the assay was 25 ng/ml; the inter- and intra-assay coefficients of variation were 7, 1, and 10.3%, respectively.

Statistical Analysis

Results are expressed as means ± SEM. The data were treated by analysis of variance (ANOVA) followed by a post-hoc Student-Newman-Keuls multiple-range test to compare group means. The level of significance was set at $p < 0.05$.

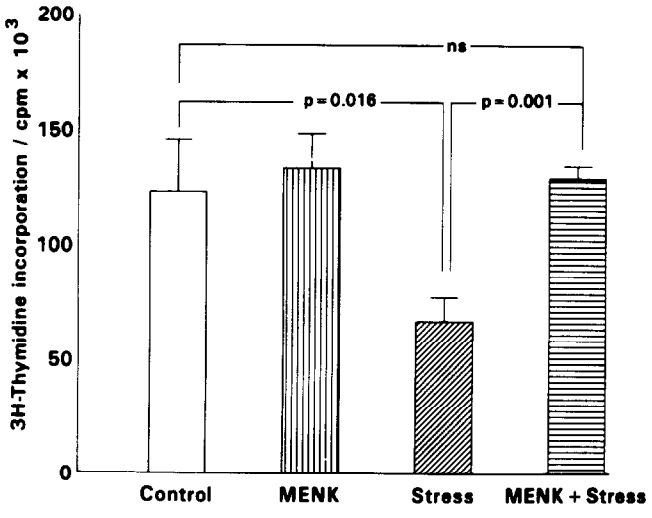

FIG. 1. Effect of met-enkephalin on the proliferative capacity of spleen cells from mice exposed to overnight restraint stress. Data were obtained from five separate experiments and are expressed as mean ± SEM (n = 8–10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. M = met-enkephalin in a single IP dose of 10 mg/kg; S = overnight restrain stress (2000–0800 h); M + S = met-enkephalin 30 min before the onset of stress [$F(3, 39) = 8.6$; $p < 0.01$].

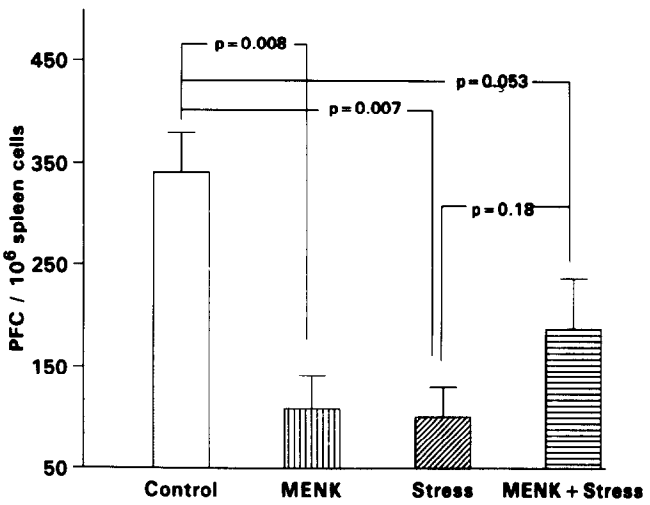

FIG. 2. Effect of met-enkephalin on plaque-forming cells (PFC) in the spleen of mice exposed to overnight restraint stress. Data are expressed as the mean ± SEM of five separate experiments (= 8–10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. For details, see Fig. 1 [$F(3, 43) = 26.1$; $p < 0.01$].

RESULTS

Effect of MENK on ConA-Driven T-Cell Proliferation in the Spleens of Stressed Mice

ANOVA revealed a significant difference between the effects of stress, MENK, or both treatments on the ConA-driven proliferation of spleen cells [$F(3, 39) = 8.6$; $p = 0.01$]. The inhibitory effect of stress ($p = 0.001$) was abolished in mice treated with MENK before stress (Fig. 1).

Effect of MENK on PFC Response in the Spleens of Stressed Mice

A group effect of stress, MENK, or both treatments on the primary antibody response to SRBC (PFC count) was observed [$F(3, 43) = 26.1$; $p = 0.01$]. Stress caused a strong inhibitory effect ($p = 0.007$); the effect of MENK injection was comparable ($p = 0.008$). Treatment of mice with MENK before stress only partially reversed the effects of stress ($p = 0.053$) (Fig. 2).

Effect of MENK on Phagocytic Activity of Splenocytes of Stressed Mice

A significant group effect of stress, MENK, or both treatments on phagocytosis was observed [$F(3, 33) = 4.52$; $p = 0.01$]. Exposure of the mice to stress increased the phagocytic ability of the spleen cells ($p = 0.001$). MENK injection did the same. However, MENK injected before stress attenuated the enhancing effect of stress ($p = 0.08$) (Fig. 3).

Effect of MENK on NK Activity of Splenocytes of Stressed Mice

A significant group effect of stress, MENK, or both treatments on the NK activity of spleen cells was observed [$F(3,$

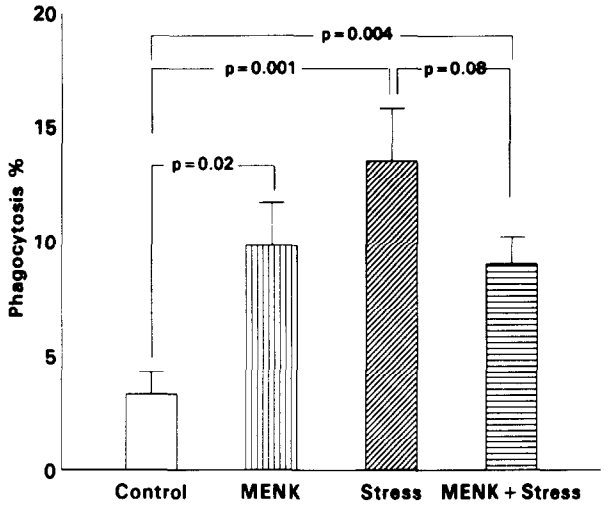

FIG. 3. Effect of met-enkephalin on the phagocytic ability of spleen cells of mice exposed to overnight restraint stress. Data are expressed as the mean ± SEM of five separate experiments (n = 8–10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. For details, see Fig. 1 [$F(3, 33) = 4.52$; $p < 0.01$].

TABLE 1

EFFECT OF MET-ENKEPHALIN ON THYMUS AND ADRENAL GLAND WEIGHT OF MICE EXPOSED TO OVERNIGHT RESTRAINT STRESS

	Thymus weight (mg)	Adrenal gland weight (mg)
Control	27.5 ± 3.3*	8.6 ± 0.6
MENK	33.0 ± 4.5	8.1 ± 0.4
Stress	20.6 ± 2.5*	8.3 ± 0.6
MENK + stress	20.5 ± 1.0*	8.3 ± 0.4

Data are expressed as mean ± SEM of five separate experiments (N = 12–15 mice per group). Nonstressed, control, animals were injected at the same time and schedule with saline. For details see legend to Fig. 1. $F(3, 61) = 18.9$; $p < 0.01$; or $F(3, 63) = 0.6$, respectively; *$p < 0.05$.

35) = 11.6; $p = 0.001$]. Restraint stress drastically suppressed NK activity ($p = 0.001$). MENK injection did the same. However, MENK injected before stress did not alter stress-induced NK inhibition (Fig. 4).

Effect of MENK on CS Levels in Plasma of Stressed Mice

A significant group effect of stress, MENK, or both treatments on plasma CS levels was found [$F(3, 38) = 4.0$; $p = 0.01$]. Exposure of mice to stress elevated the CS levels in plasma ($p = 0.04$). MENK did the same ($p = 0.001$). With MENK injected before stress, the CS level remained on the control level (Fig. 5).

Effect of MENK on Organ Weight and/or Cellularity in Stressed Mice

Stress, MENK, or both treatments caused a significant group effect on the weight [$F(3, 33) = 10$; $p = 0.001$] and cellularity of the spleen [$F(3, 33) = 3.2$; $p = 0.03$] and thymus [$F(3, 61) = 18.9$; $p = 0.01$]. The effect on the adrenal glands was not significant [$F(3, 63) = 0.6$] (Table 1). In contrast to the stress-induced loss of spleen weight ($p = 0.003$), the cellularity of the spleen was increased ($p = 0.02$) (Fig. 6). MENK injected before stress did not alter stress-induced weight loss (Fig. 6), but reversed the stress-induced cellularity increase. MENK itself did not affect the weights of the spleen, thymus, and adrenal glands, or spleen cellularity (Fig. 6).

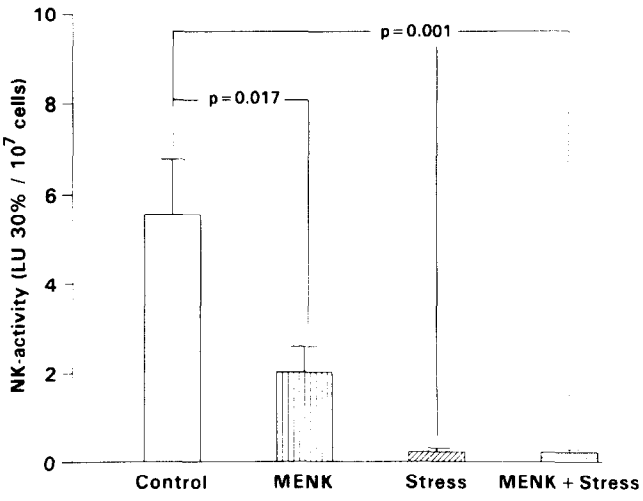

FIG. 4. Effect of met-enkephalin on the natural killer (NK) activity of mice exposed to overnight restraint stress. Data are expressed as the mean ± SEM lytic unit (LU) 30/10^7 spleen cells) of five separate experiments (n = 8–10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. For details, see Fig. 1 [$F(3, 35) = 11.6$; $p < 0.001$].

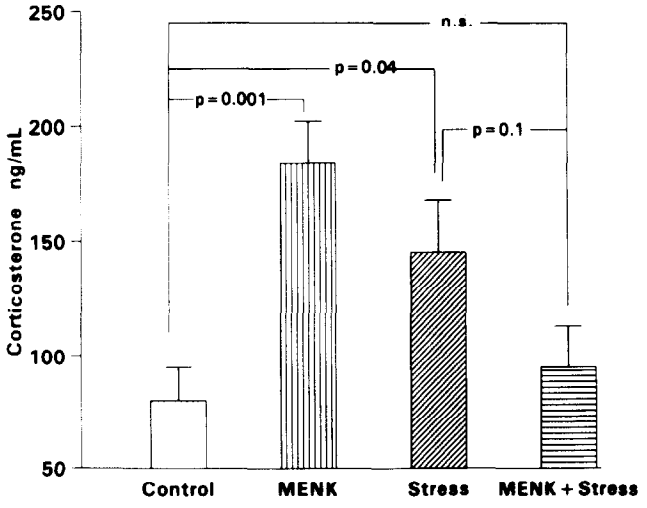

FIG. 5. Effect of met-enkephalin on the corticosterone level in the plasma of mice exposed to overnight restraint stress. Data are expressed as the mean ± SEM of five separate experiments (n = 8–10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. For details, see Fig. 1 [$F(3, 38) = 4.0$; $p < 0.01$].

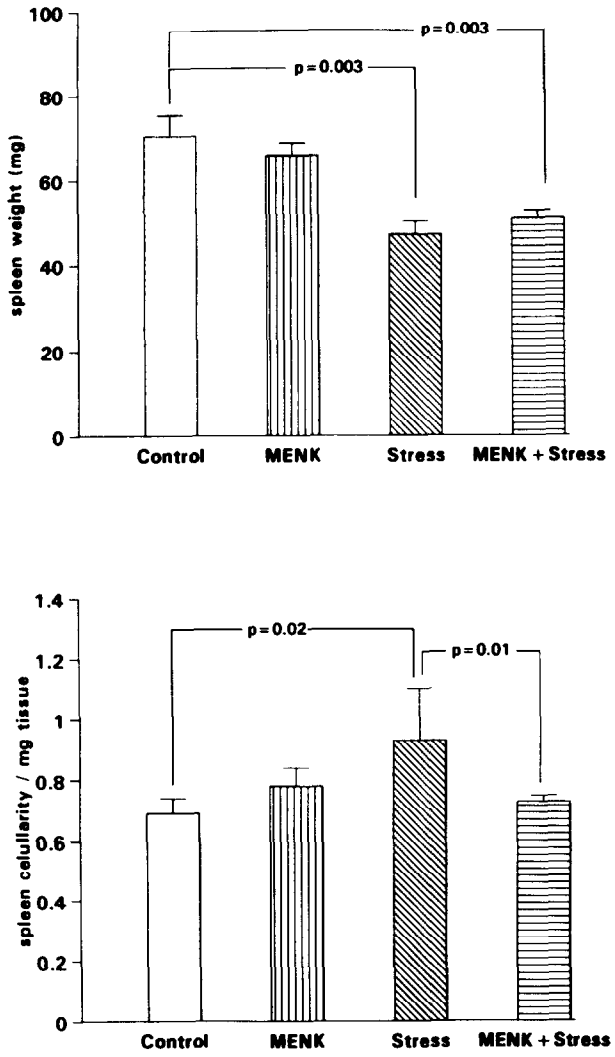

FIG. 6. Effect of met-enkephalin on spleen weight/body weight and cellularity of the spleen of mice exposed to overnight restraint stress. Data are expressed as the mean ± SEM of five separate experiments (n = 8-10 mice per group). Nonstressed control animals were injected with saline at the same time and schedule. For details, see Fig. 1 [$F(3, 33) = 10.0, p < 0.01$; and $F(3, 33) = 3.2, p = 0.03$, respectively].

Reversion of Stress-Induced Alterations of the Immune Response by Naloxone

Naloxone (10 mg/kg) injected 30 min before restraint reversed the stress-induced decrease of T-cell proliferation and the enhancement of phagocytosis (Fig. 7a and 7b). Stress-induced decreases of NK activity and the primary antibody response to SRBC were not altered (data not shown). Naloxone did not prevent stress-induced CS elevation and even showed a tendency to enhance it (Fig. 7c). By itself, naloxone did not affect either parameter (Fig. 7).

DISCUSSION

Intraperitoneal injection of endogenous opioid peptide MENK into mice changed the immune response in a manner similar to restraint stress: The PFC response to SRBC and the cytotoxic activity of the NK cells were suppressed and the phagocytic activity enhanced. This observation confirms our previous findings (30) with the exception that there was no effect of MENK on ConA-driven cell proliferation.

The injection of MENK, like exposure to stress, resulted in elevated plasma CS level. That suggests similar (or common) mechanism(s) of action. Indeed, it has been demonstrated that morphine and met- and leu-enkephalin in vitro and in vivo stimulate the secretion of CS, ACTH, and ACTH-releasing factor (CRF), and their effect could be competitively antagonized by naloxone (6). The in vivo administration of high doses of naloxone to rats produced a dose-dependent increase in plasma CS levels (12). Moreover, naloxone has a direct

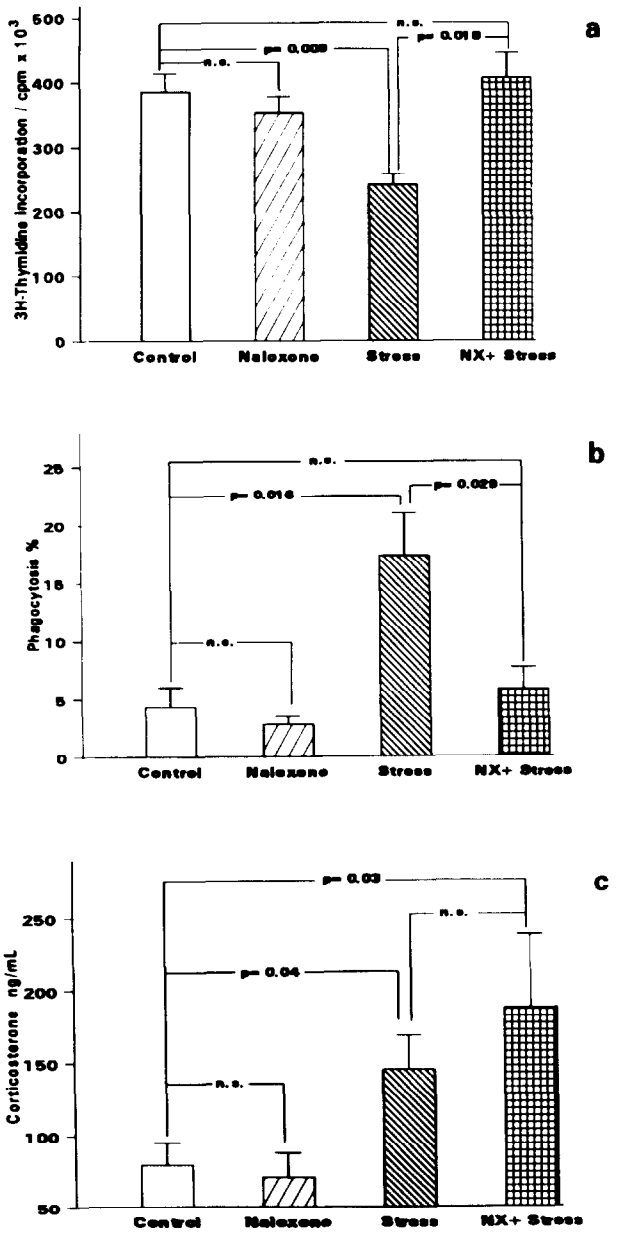

FIG. 7. Effect of naloxone on stress-induced alterations of immune responses and corticosterone. Data are expressed as mean ± SEM (n = 6-10 mice per group). Naloxone was given in a single IP dose of 10 mg/kg at the same time and schedule as MENK.

effect on the adrenal cortex in that it produced a decline in basal steroidogenesis at high, and elevation of CS secretion in lower doses (28). Intraventricular administration of β-endorphin, MENK, and morphia induce circadian prolactin release, the rhythm of which was lost in adrenalectomized animals (26). On the contrary, morphine-induced prolactin release (17) and β-endorphin–induced cortisol release were decreased in stressed animals (11).

The mechanism(s) of stress-induced suppression of the immune responses have been amply studied. It has been proposed recently that different mechanisms mediated the suppressive effects in different lymphoid cell compartments of rats exposed to foot-shock stress (9). Elevated CS in plasma was responsible for the suppression of the peripheral blood lymphocyte function (as the suppression could be reversed by adrenalectomy), and peripheral release of catecholamine was responsible for the suppression of the spleen cell functions (as it could be abrogated by β-adrenoceptor antagonists). Different mechanisms have been proposed for morphine-induced immunosuppression: CS may mediate the suppression of the peripheral blood lymphocytes; catecholamines might do so in the spleen; and there is no effect on lymphocytes in the lymph nodes (16). Studying the mechanisms(s) of action of an endogenous opioid, MENK, we have found both glucocorticoid-dependent and glucocorticoid-independent actions (30). MENK effects on T- and B-cell functions were absent in adrenalectomized mice, whereas the suppressive effect on NK cells was still present. Thus, even in the same lymphoid compartment, different cell types are affected differently by MENK. Likewise β-adrenoceptor antagonists fully reverse the suppression of spleen T-cell functions in rats after morphine administration, but cannot reverse concomitant suppression of NK activity (16). Morphine-induced suppression of NK activity seems to be mediated via μ-2-opioid receptors in the brain (central neuroendocrine mechanisms) and by α-adrenoceptors in peripheral regulation as judged by its blockade by specific antagonists in a dose-dependent manner (8).

If MENK and stress share common mechanism(s) by which the immune functions are modulated, pretreatment with MENK should modify the effects of stress. Indeed, MENK injected before stress reversed or attenuated the stress-induced suppression of T-cell proliferation and of the antibody response. Concomitantly with normalization of the immune functions, plasma CS levels also returned to a normal range. That observation additionally supports the idea that the activation of the HPA axis could be the common mechanism of stress and MENK-induced immunomodulation. Indeed, MENK-induced suppression of antibody response and T-cell proliferation is abrogated by adrenalectomy (30).

The stress-induced suppression of NK cytotoxicity could not be reversed by MENK. This is in contrast to the reversion of stress-induced suppression of T-cell proliferation (completely) and antibody production (partially), as well as stress-induced enhancement of phagocytic activity (partially). The inability of MENK fully to reverse the stress-induced decrease of PFC may simply reflect the involvement of different types of cells which are distinctively regulated by MENK/stress treatment (T- and B-lymphocytes vs. phagocytes). Opposite alterations of T- and B-lymphocyte functions vs. NK cell activity in the spleens of mice exposed to stress or MENK suggest the involvement of different mechanisms of action, which depend on the cell type involved.

Unlike lymphocyte proliferation and antibody production, which were suppressed by stress, the phagocytic activity of spleen cells was stimulated. MENK injected before stress abrogated the stress-induced enhancement of phagocytosis, although injected alone it caused stimulation, like stress. The increased phagocytic activity of polymorphonuclear (PMN) leukocytes has been observed in rats exposed to repeated foot-shock. Stress also increased IL-1 secretion by peritoneal exudate cells (25,41). Factors which enhance the activity of phagocytic cells in stressed animals are not known, but may include the stress-induced secretion of endogenous opioid peptides such as β-endorphin (37). The data of our study support that idea, as MENK injection enhanced the phagocytic activity of spleen cells. Another recent study (29) has shown a direct stimulatory effect of MENK on the phagocytic activity of mouse spleen cells. Macrophages are considered to be the primary targets for opioid action, resulting in the enhanced secretion of several mediators (20). Differences in noradrenergic innervation of lymphoid vs. myeloid areas of the spleen (18), and/or the expression of the respective receptors (19) may account for the observed distinctions between lymphocyte and macrophage functions caused by stress and/or opioid action. Another possible explanation for stress and MENK-induced enhancement of phagocytosis could be the result of hormones other than CS, such as growth hormone (GH) or prolactin (PRL). Indeed, exogenous opioids (17) and stress (39) induce the secretion of GH and PRL; pituitary grafts placed under the kidney capsule of hypophysectomized rats are capable of restoring rats' immunocompetence (2); and, moreover, treatment of mice with drugs stimulating the release of PRL can antagonize the suppression of immune responses induced by glucocorticoid treatment (3).

Stress and MENK elevated the CS level, although without significantly increasing the weight of the adrenal glands. In mice and rats, respectively, a strong reduction of thymus and spleen weight was observed after a 15-h restrain stress (44) with no further reduction upon more prolonged stress (1). Morphine had the same effect on thymus and spleen weight but additionally elevated adrenal weight (5). An absence of a significant effect of MENK on thymus weight in this study, compared to the earlier reported MENK-induced decrease of thymus weight (24), may reflect the mode of application (single vs. multiple MENK) and shorter exposure (12 h vs. 4 days).

A striking similarity in the alterations of the immune reactivity induced by stress and MENK suggests that the stress-induced opioids may contribute to the observed alterations of the immune responses. Pretreatment of mice with the opioid receptor antagonist naloxone (10 mg/kg, IP, 30 min before restraint) abrogated the stress-induced alterations of phagocytic activity and of T-cell proliferation. However, NK activity and PFC response remained low. The inability of naloxone to reverse stress-induced suppression of those functions need not exclude the role of the opioids (MENK itself was suppressive), but may suggest the operation of other mechanism(s), such as a direct activation of the HPA axis. The inability of naloxone to abolish the elevation of the CS level is in accordance with that idea.

Thus, immunosuppression induced by restraint stress seems to be mediated by more than one mechanism. The possibilities include activation of the HPA axis and the induction of EOP secretion. EOP, in turn, may also activate the HPA axis or directly affect the immunocytes. The relative contribution of these mechanisms to the functional alterations probably vary with the cell type involved. Additional regulatory mechanism(s) (e.g., catecholamines) may also be involved.

Collectively, the data show similar alterations of immune functions by restraint stress and by the opioid peptide MENK. Furthermore, MENK was able to reverse stress-induced alterations of phagocytic and lymphocyte functions, except for NK cell activity. The interaction of MENK and stress may occur at the level of the HPA axis, or by direct interference of the exogenously applied and endogenous stress-induced opioid peptides.

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