Research report

Intracerebroventricular met-enkephalin administration modulates adjuvant arthritis


Abstract

The purpose of this study was to investigate the anti-inflammatory properties of intracerebroventricular met-enkephalin (met-enk) administration in an animal model of arthritis. Adjuvant arthritis was induced in rats by intradermal inoculation of Mycobacterium butyricum and the effects of intraventricular met-enk + thiorphan (enkephalinase inhibitor) were studied. Treatment was initiated either simultaneously with the bacterial inoculation (preventive group) or on post-inoculation day 17 after the appearance of inflammation (treatment group). The degree of inflammation was evaluated by measuring the diameter and the circumference of the ankle joint immediately before the sacrifice (day 31) and by histologic examination of ankle joint sections. The results of this study revealed that combined intraventricular injections of met-enk + thiorphan reduced the arthritic-like inflammation in the preventive group as well as in the treatment group. These findings suggest that centrally applied met-enk + thiorphan may suppress the development adjuvant arthritis as well as the symptoms of manifest arthritis. Thus central met-enk may be involved in both hypothalamic pituitary adrenal axis and immune forms of stress-induced modulation.

Theme: Neurotransmitters, modulators, transporters, and receptors

Keywords: Met-enk; Synovial cells; Ankle; Mycobacteria; Arthritis

1. Introduction

Adjuvant arthritis is an experimentally inducible form of arthritis in the rat that shares many features with human rheumatoid arthritis [25]. Although the immune system has long been thought to be involved in its pathogenesis [14,15], the nervous system may also play a role in the development of this disease [18]. Methionine-enkephalin (met-enk) is an endogenous opioid that is present both in the central and peripheral nervous systems [33]. Receptors for met-enk have been detected in immune cells and peptide synthesis has been observed in activated T-cells [37,39]. In vitro studies have revealed that met-enk modulates a number of immune functions such as natural killer cell activity, antibody production, lymphocyte proliferation and macrophage activity [30]. In vivo studies revealed that met-enk administered intraperitoneally in the rat either increased or decreased the plaque-forming cell response and IgM production depending upon the dose used [16]. Moreover, peripherally administered met-enk has also been shown to affect IgG antibody production and hypersensitivity skin reactions [21]. That met-enk may act through central mechanisms to modulate immune responses is indicated by the following observations. Intracerebroventricular (ICV) administration of met-enk...
modulates the cutaneous immune response in rats [35], decreases the clinical manifestations of experimental allergic encephalomyelitis and markedly attenuates the magnitude of inflammatory brain lesions [34]. The purpose of the present investigation was to examine the effects of centrally administered met-enk on adjuvant induced arthritis in the rat. For these experiments, rats were given met-enk ICV in a concentration of 20 µg which has been shown to reduce the inflammatory response to peripherally administered carrageenin [5]. In addition, the enkephalinase inhibitor, thiorphan was given in some animals to potentiate the effects of exogenously administered met-enk.

2. Materials and methods

The study included thirty-three 10-week-old female Lewis rats with an initial body weight between 160 and 180 g. The animals were housed singly at 21°C on a 12 h light/dark cycle and fed ad libitum with a standard pellet diet and water. The experiments were approved by the regional ethical committee, Stockholm, Sweden.

2.1. ICV cannulation

The surgical procedure of cannulation was performed as follows. The rats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (60 mg/kg body weight i.p., Mebumal, Nord vacc, Stockholm, Sweden). The head was fixed in a stereotaxic frame and a mid-line skin incision was made from bregma to lambda and the connective tissue over the periosteum was removed. A single guide cannula (28-gauge stainless-steel, 9.5 mm long) was implanted just above the right lateral ventricle (coordinates: 1.5 cm caudally, 1.5 laterally and 3.5 ventrally from the bregma). Three stainless steel screws fixed to the skull and cannulae were fixed in place by dental cement. The drugs were injected slowly through a 28-gauge stainless-steel guide cannula (Plastic Product Inc., Roanoke, NJ, USA). Animals were allowed at least 7 days to recover prior to the initiation of experiments. During the recovery period, the rats were handled for about 10 mm/day for at least 5 days to familiarize them with infusion procedures.

2.2. Substances

The commercial preparation of met-enk and thiorphan used for this study were purchased from BACHEM Feinchemikalien AG, Switzerland. The drugs were dissolved in artificial cerebrospinal fluid (ACSF) consisting of: sodium chloride, 8.98 g; potassium chloride, 0.25 g; calcium chloride, 0.14 g; magnesium chloride, 0.11 g; sodium dihydrogen phosphate, 0.07 g; urea, 0.13 g; and glucose 0.61 g dissolved in triple-distilled water to give 1000 ml. The vehicle group received ACSF only.

2.3. Induction of arthritis

Arthritis was induced in 21 rats by intradermal injection of a suspension (50 µl) of heat-killed Mycobacterium butyricum in paraffin oil (10 mg/ml) into the base of the tail [25]. The sham group (seven animals), serving as controls, received 50 µl paraffin oil via the same route.

2.4. Drugs administration

The animals were randomly assigned to five treatment groups of seven rats each (except for naive), as follows: (1) The rats of the naive group (five rats) were unoperated and received no intradermal injections; (2) The rats of the sham group were fitted with an ICV cannula but received no intradermal injection; (3) The rats of the vehicle group were cannulated and received a suspension of heat-killed M. butyricum in paraffin oil (50 µl) and an ICV injection of 40 µl of ACSF 30 min before inoculation daily for 14 days; (4) The rats of the preventive study group received daily injections of met-enk (20 µg) + thiorphan (200 µg) into the right lateral ventricle 30 min before inoculation of mycobacteria over the 14 day administration period; (5) The rats of the treatment group which received mycobacteria inoculations for 14 days, received injections of met-enk (20 µg) + thiorphan (200 µg), in the right lateral ventricle on day 17 through day 30.

Prior to sacrifice, the extent of the inflammatory reaction was evaluated by tape measuring the circumference of the ankle joint in all animals. The diameter of the right and left ankles were measured in the frontal plane at the level of the maleoli using vernier calipers. All the animals were killed on day 31. Animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone (60 mg/kg body weight) and perfused intra-arterially with phosphate buffer saline (PBS), followed by Zamboni’s buffered paraformaldehyde solution containing 0.2% picric acid [19]. The ankles were excised and immersed in Zamboni’s fixative for 2 days at +4°C. The specimens were de-mineralized in a 4% ethylene-diamino-tetraacetic acid (EDTA) solution at pH 7.3 for 3 weeks on average [6]. The ankles were blocked in paraffin and cut with a microtome. Multiple 5-µm sections were obtained and stained with hematoxylin and eosin (H&E). Slides were examined by light microscopy by the two pathologist (N.P and A.G.), who were unaware of the experimental group from the tissue had been obtained. At least three sections were evaluated from each animal. A system was used to score the H&E-stained slides to reflect the magnitude of thickening of the diffuse synovial membrane (<5 cell layers thick = 0; 5–10 cell layers = 1; 10–15 cell layers = 2; >15 cell layers = 3), the presence of subsynovial cells, the degree of inflammatory leukocytic cell infiltration (mono-
nuclear cells and polymorphonuclear leukocytes), the presence of follicles, detection of granuloma and others factors such as the presence of abscess and necrosis.

2.4.1. Statistical analysis
Comparisons of different treatment conditions on ankle circumference and diameter were performed by one-way ANOVA followed by Fisher’s protected LSD analysis. For all comparisons, \( P<0.05 \) was considered as significant.

3. Results

3.1. Clinical evaluation

Clinical signs of arthritis were evident on average by day 10 post inoculation of mycobacteria. The signs persisted until the end of the experiment. X-ray films of the ankle joints from vehicle-treated rats on day 30 confirm the occurrence of arthritis. No sign of inflammation was noted in the naive and sham groups while the other groups showed variable degrees of inflammation.

The mean diameter of the ankle joints of the vehicle group was significantly greater than that found in the control and naive groups (Fig. 1). However, there was no significant difference between the mean diameter of the ankle joints of the preventive and the treatment conditions compared to the naive or the sham groups. A significant difference was observed between the vehicle group and the preventive and the treatment groups (Fig. 1).

The mean ankle joint circumference of the vehicle, preventive and treatment groups were significantly greater compared to the naive and sham groups (Fig. 2). Moreover, there was a significant difference between the preventive and the vehicle groups, but no significant difference between the vehicle and the treatment groups (Fig. 2). There was no significant difference between the circumference and diameter of the right versus left ankle joints in the individual rat.

3.2. Histologic findings

Histological examination performed on ankle joints collected from the animals on post inoculation day 31 showed that the injection of mycobacteria was capable of inducing arthritis. Arthritic-like symptoms included synovial hypertrophy, pannus formation, neovascularization and synovial infiltrates comprised predominantly of mononuclear inflammatory cells, some polymorphonuclear leukocytes, and a the presence of a few eosinophils.

Histological examination of synovial lining, subsynovial cells, leucocytic infiltration, follicular, granuloma, necrosis and abscess formation revealed that groups were affected differentially. With regard to synovial lining hypertrophy, the vehicle group was most affected while the treatment group and the preventive group were affected the least (Table 1). Subsynovial cell infiltrates (Table 2) and

<table>
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<tr>
<td>Ankle joint histologic sections from different treatment groups assigned synovial lining score*</td>
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<tr>
<td>Group</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>Synovial lining</td>
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<tr>
<td>Naive</td>
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<td>Sham</td>
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<td>Vehicle</td>
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<td>Enk preventive</td>
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<td>Enk treatment</td>
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* Each value corresponds to the number of animals and the percentage in (brackets) showing the specific score for each group.
Table 2

<table>
<thead>
<tr>
<th>Group</th>
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<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td>Subsynovial cells</td>
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<tr>
<td>Naive</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>7 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>1 (14%)</td>
<td>3 (43%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>Enk preventive</td>
<td>5 (71%)</td>
<td>2 (29%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enk treatment</td>
<td>3 (43%)</td>
<td>3 (43%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
</tbody>
</table>

*Each value corresponds to the number of animals and the percentage in (brackets) showing the specific score for each group.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
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<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
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<tr>
<td>Leucocytes</td>
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</tr>
<tr>
<td>Naive</td>
<td>5 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>7 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Vehicle</td>
<td>2 (29%)</td>
<td>2 (29%)</td>
<td>3 (42%)</td>
<td>0</td>
</tr>
<tr>
<td>Enk preventive</td>
<td>5 (71%)</td>
<td>2 (29%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enk treatment</td>
<td>5 (72%)</td>
<td>1 (14%)</td>
<td>0</td>
<td>1 (14%)</td>
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</table>

*Each value corresponds to the number of animals and the percentage in (brackets) showing the specific score for each group.

leucocytic infiltration (Table 3) were most apparent in the vehicle group followed by the treatment group and the preventive groups.

Tissues from animals in the preventive or treatment conditions did not contain follicles, granulomas or areas of necrosis. However, follicles were observed in tissues derived from five rats of the vehicle group. Three vehicle animals also displayed granulomas and one showed necrosis (results not shown). The total mean scores from the total evaluation of the ankle joints histopathology parameters for each treatment group is summarized in Fig. 3.

4. Discussion

The results of this study illustrate two important points concerning the effects of intraventricular met-enk in the development and treatment of adjuvant arthritis. Firstly, exogenous met-enk modulates the development of adjuvant arthritis. All rats developed less severe clinical and histopathological signs of adjuvant arthritis when the peptides were given as a preventive drugs. Secondly, ICV injection of met-enk attenuates the development of adjuvant arthritis and reduces the appearance of both clinical and histopathological signs of the disease.

Met-enk in the brain is rapidly degraded by multiple enkephalin-degrading enzymes [17]. Thiorphan is known as an enkephalinase inhibitor and extends the half-life of enkephalins [27]. At the concentration used in this study, no other effect of thiorphan has been described on the literature. Roques et al. observed that met-enk administered intracerebro-ventricularly which alone had no significant effect on the tail-withdrawal test, resulted in strong and long lasting analgesia when administered along with thiorphan. Thiorphan administered alone elicited a clear antinociceptive activity [7].

Using a range of concentrations of thiorphan, Yaksh and Harty showed that thiorphan (200 µg) potentiated the effects of met-enk [38]. In agreement with this finding, Malin et al. used 250 µg thiorphan ICV to augment transcranial electrostimulation analgesia [20]. In a related model, Bhattacharya and co-workers ICV administration of met-enk triggers a variety of neurohormonal effects, some of them related to the pathogenesis of inflammation [5].

The median eminence is rich in opioid receptors [1]. Many studies showed that met-enk has major effects on the release of many pituitary hormones including adrenocorticotropic hormone (ACTH) [9,11], growth hormone [8], prolactin [8], thyrotropin [23] and luteinizing hormone [8]. In the hypothalamus enkephalin immunoreactivity has been localized in the parvocellular neurons of the hypothalamic paraventricular nucleus which projects on to the median eminence [13], the site where hypothalamic releasing factors are secreted into the portal blood, hence the anterior pituitary gland.

The female Lewis rat is susceptible to adjuvant arthritis after inoculation of mycobacteria. In contrast, the histocompatible female Fisher rat develops only a mild transient acute arthritis. Steinberg and colleagues showed that compared to Fisher rats, the female Lewis rat displays a reduction in glucocorticoid release in response to injections of streptococcal cell wall peptidoglycan and that these deficits are due to reductions in hypothalamic CRH synthesis and release [31,32]. It is interesting to note that deficits in CRH production occur together with the reductions in the expression of the hypothalamic enkephalin gene [32]. It is possible that the administration of met-enk in Lewis rats may restore the hypothalamic function in regulating the pituitary hormones release. Corticosteroids
are both potent endogenous anti-inflammatory and immunosuppressive agents. In addition, growth hormone and prolactin are important factors in the development of adjuvant arthritis since hypophysectomized rats fail to develop arthritis unless exogenous growth hormone or prolactin are provided [2]. In the clinic, changes in prolactin and growth hormone concentrations profoundly affect the manifestation of adjuvant arthritis [2].

It is also possible that exogenous met-enk affects adjuvant arthritis through an action on immune cells. Immunomodulation is well known during stress which is mediated centrally via opioid and non-opioid mechanisms [28]. Stressors such as inflammation are associated with increased level of endogenous opioid in the brain [22]. This condition can be simulated by the administration of exogenous opioid ICV which may alter specific functions. For example, immune functions are affected by opioids either via a direct action on immune cells, such as lymphocytes, monocytes, splenocytes and thymocytes or by acting in the brain [10,26]. Central actions result in increased level of endogenous opioid in the brain [22].

Impaired immune function has been observed in opioid addicts and was confirmed by in vivo and in vitro studies demonstrating immunosuppressant actions of opioids [10,12,36]. More recently, opioids applied centrally were able to regulate IL-6 production [3,4]. Our results show that ICV application of exogenous met-enk together with thiorphan leads to an attenuation of the development of adjuvant arthritis and a suppression in the development of arthritic symptoms at both the macroscopic and histopathological levels. Further experiments are necessary to determine the optimal concentrations of met-enk and thiorphan as well as naloxone to be employed in this paradigm. However, the present results clearly suggest that increased ICV concentrations of met-enk can diminish this form of arthritis.

References

[15] J. Holoshitz, Y. Naparstek, A. Ben-Nun, I.R. Cohen, Lines of that ICV application of exogenous met-enk together with thiorphan leads to an attenuation of the development of adjuvant arthritis and a suppression in the development of arthritic symptoms at both the macroscopic and histopathological levels. Further experiments are necessary to determine the optimal concentrations of met-enk and thiorphan as well as naloxone to be employed in this paradigm. However, the present results clearly suggest that increased ICV concentrations of met-enk can diminish this form of arthritis.

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