

Alteration in endogenous opioid systems due to chronic inflammatory pain conditions

Mariana Spetea^{a,*}, Gustav Rydelius^a, Ingrid Nylander^b, Mahmood Ahmed^a,
Indre Bileviciute-Ljungar^c, Thomas Lundeborg^c, Stefan Svensson^d, Andris Kreicbergs^a

^aSection of Orthopaedics, Department of Surgical Sciences, Karolinska Institute, Orthopaedic Research Laboratory M3:02, Karolinska Hospital, S-171 76 Stockholm, Sweden

^bDepartment of Pharmaceutical Biosciences, Division of Pharmacology, Uppsala University, S-751 24 Uppsala, Sweden

^cDepartment of Physiology and Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden

^dSection of Bacteriology, Department of Veterinary Microbiology, POB 7036, SE-750 07 Uppsala, Sweden

Received 24 September 2001; received in revised form 19 November 2001; accepted 23 November 2001

Abstract

The influence of chronic arthritic pain on two endogenous opioid peptides, dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷, and multiple opioid receptors in discrete brain, lumbar spinal cord and pituitary pools was investigated. Using radioimmunoassay and receptor binding assay, we examined the changes in regional opioid peptide levels and opioid receptor activity due to chronic inflammation in adjuvant arthritic rats. At 4 weeks post-inoculation, increased levels of immunoreactive dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ were measured in tissues of arthritic rats compared with controls. No significant changes in μ -, δ - or κ -opioid receptors were seen after chronic inflammation. Taken together, these results indicate that in chronic arthritis, opioid receptor changes do not follow the peptide alterations of pro-dynorphin and pro-enkephalin systems. Thus, dynamic modification and modulation of nociceptive information takes place during chronic inflammation. This supports the key role of the central nervous system in chronic inflammatory pain conditions. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Opioid peptide; Opioid receptor; Adjuvant arthritis; Inflammation; Chronic pain; Central nervous system

1. Introduction

Two advances have increased our understanding of the role of the endogenous opioid system in the modulation of nociception in the central and peripheral nervous systems: (i) the characterisation of opioid receptors, and (ii) the identification of opioid peptides (Simon and Hiller, 1994). The opioid system involves at least three main receptor classes: μ , δ and κ , that differ in their ligand selectivity and anatomical distribution. Endogenous opioid peptides are classified into three families: β -endorphin (derived from pro-opiomelanocortin), [Met⁵]- and [Leu⁵]enkephalins (from pro-enkephalin) and the dynorphins (arising from pro-dynorphin). These peptides have distinct but overlapping distributions in the

brain (Mansour et al., 1988). β -endorphin interacts predominantly with μ - and δ -opioid receptors, [Met⁵]- and [Leu⁵]enkephalins act mainly on δ -opioid receptors, while dynorphins show preference for κ -opioid receptors. Two additional endogenous opioid peptides, with high affinity and selectivity for μ -opioid receptors, have been isolated from bovine and human brain, endomorphin 1 and endomorphin 2 (Zadina et al., 1997), although their precursors are not known yet.

There is considerable evidence that the functional activity of endogenous opioid systems is enhanced by the acute imposition of noxious stimuli (Besse et al., 1992; Hong and Abbott, 1994; Ji et al., 1995; Bileviciute-Ljungar and Spetea, 2001). In the spinal cord, it was demonstrated that both pro-enkephalin and pro-dynorphin opioid systems are affected by acute noxious stimuli, such as carrageenan-, formalin- and Freund-adjuvant acute-induced inflammation. Such painful stimuli profoundly enhanced pro-dynorphin and to a lesser extent pro-enkephalin biosynthesis in spinal and supraspinal neurons (Idarola et al., 1988; Noguchi et al., 1989). This increase was found to be paralleled by an

* Corresponding author. Present address: Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria. Tel.: +43-512-507-5606; fax: +43-512-507-2931.

E-mail address: Mariana.Spetea@uibk.ac.at (M. Spetea).

increase in the mRNAs encoding pro-dynorphin and pro-enkephalin (Hölldt et al., 1987; Idarola et al., 1988).

It has emerged that chronic exposure may also cause a long-term, sustained influence upon particular opioid networks (Millan et al., 1986, 1987; Hölldt et al., 1987; Stein et al., 1988). Currently, the most thoroughly characterised model available of chronic inflammatory pain is that of polyarthritis in the rat, which represents a generalised disease state affecting many tissues and systems (Colpaert, 1987). It has been reported that polyarthritic rats display a pronounced increase in the levels and synthesis of dynorphin B in the lumbar spinal cord, which might play a role in the modulation of nociception under chronic pain (Millan et al., 1987).

Although several data indicate that the activity of certain opioid systems is profoundly modified under chronic arthritic pain, there remain a number of fundamental questions regarding the nature of these changes.

To further analyse the changes of central pain modulatory systems under pathological conditions and to determine their potential role in the pathophysiology of pain syndromes, in the present study, we have focused on the representatives of two families of endogenous opioid peptides, dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷, derived from the pro-dynorphin and the pro-enkephalin, respectively. In addition to the examination of these two peptides, we have also characterised the influence of chronic arthritis on multiple opioid receptors in discrete brain, lumbar spinal cord and pituitary pools. Using radioimmunoassay and receptor binding assay, we assessed the changes in regional opioid peptide levels and opioid receptor activity due to chronic inflammation in adjuvant arthritic rats.

2. Materials and methods

2.1. Animals

Female Lewis rats (M&B, Denmark) weighing 200 g were used. The animals were housed in groups of six per cage at 21 °C in a 12-h light/dark cycle with free access to food and water according to the Karolinska Institute protocol. All experiments were approved by the Ethics Committee for Animal Research, Stockholm North.

To induce adjuvant arthritis, rats were anaesthetised with methoxyflurane (Schering-Plough Animal Health, Union, NJ, USA) and inoculated by intradermal injection into the base of the tail of a suspension (50 µl) of heat-killed *Mycobacterium butyricum* in paraffin oil (10 mg/ml) (Ahmed et al., 1995a). Non-arthritic control animals received similar injections with 50 µl paraffin oil.

Both control and arthritic rats were killed by decapitation 29 days after adjuvant or vehicle injection. The brain was immediately removed and dissected on ice to yield the striatum, hippocampus, hypothalamus, frontal and occipital cortices. The pituitary gland was divided into anterior and neurointermediate lobes. The lumbar part of the spinal cord

was also dissected. Spleen and thymus were removed from the body and weighed. Following dissection, tissues were immediately frozen on dry ice and stored at –70 °C until further analysis. The diameters of left and right ankle joints were measured with a caliper.

2.2. Radioimmunoassay

Tissue extractions were performed in 1 M acetic acid. Samples were boiled for 10 min, cooled on ice and then homogenised, sonicated (30 s) and centrifuged at 3000 × g for 15 min. The supernatants were lyophilised, diluted in 2 ml of 0.05 M phosphate buffer (pH 7.4) and kept at –20 °C until analysis. Peptides were analysed with specific radioimmunoassays according to previously described procedures (Christensson-Nylander and Terenius, 1985; Ploj et al., 2000). The samples and standard peptides were incubated with rabbit antiserum and ¹²⁵I-labeled peptides for 24 h at 4 °C. The samples and standard peptides were subjected to oxidation prior to analysis in the [Met⁵]enkephalin-Arg⁶-Phe⁷ assay. The samples were dissolved in 1 M acetic acid and 35% hydrogen peroxide, incubated at 37 °C for 30 min and then dried in a vacuum centrifuge. The [Met⁵]enkephalin-Arg⁶-Phe⁷ antiserum (90:3D) was used in a final dilution of 1:140,000 and the dynorphin B antiserum (113B) of 1:562,500 in the respective assay. In the dynorphin B assay, separation of the antibody-bound and free peptides was performed by adding a suspension of sheep antirabbit antiserum (Pharmacia Decanting Suspension 3, Pharmacia Diagnostics, Uppsala, Sweden), and subsequently, the samples were incubated for 1 h. In the [Met⁵]enkephalin-Arg⁶-Phe⁷ assay, a charcoal suspension was added, followed by incubation for 10 min. After centrifugation, the radioactivity was determined in the residual pellets (dynorphin B assay) or supernatant ([Met⁵]enkephalin-Arg⁶-Phe⁷ assay) using a γ-counter (Wallac, Turku, Finland).

2.3. Receptor binding assay

Membranes were prepared as previously described (Spetea et al., 1998). Briefly, tissues were homogenised in 5 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4). After centrifugation at 40,000 × g for 20 min at 4 °C, the membrane pellets were resuspended in 30 volumes of Tris–HCl buffer and incubated at 37 °C for 30 min. The centrifugation step described above was repeated, the final pellet was resuspended in 5 volumes of 50 mM Tris–HCl buffer (pH 7.4) and stored at –70 °C until use.

Binding experiments were performed in 50 mM Tris–HCl buffer (pH 7.4) in a final volume of 0.25 ml containing 0.1–0.2 mg protein. The following radioligands were used: [³H]DAMGO ([³H]D-Ala²,Me-Phe⁴,Gly-ol⁵]enkephalin, 54 Ci/mmol, New England Nuclear, Boston, MA, USA), [³H][Ile^{5,6}]deltorphin II (30 Ci/mmol, Isotope Laboratory of the Biological Research Centre, Szeged, Hungary) (Nevin et al., 1994) and [³H]U69,593 ([³H]5α,7α,8β-(–)-N–

Table 1
Influence of adjuvant arthritis upon various parameters in the rat

	Body weight (g)	Ankle joint diameter (mm)	Spleen weight (g)	Thymus weight (g)
Control	200.6 ± 2.3 (10)	5.27 ± 0.05 (10)	577 ± 22 (10)	215 ± 9 (10)
Arthritic	186.9 ± 2.2 (9) ^a	7.97 ± 0.37 (9) ^b	679 ± 28 (9) ^a	165 ± 10 (9) ^a

Values represent means ± S.E.M.; *n* is in parentheses.

^a Significance of arthritic vs. control animals: $P < 0.01$ (Student's two-tailed *t*-test).

^b Significance of arthritic vs. control animals: $P < 0.001$ (Student's two-tailed *t*-test).

methyl-*N*[7-(1-pyrrolidinyl)-1-oxaspiro(4–5)dec-8-yl]benzeneacetamide, 47 Ci/mmol, New England Nuclear) (Lahti et al., 1985). Homogenates were incubated with either the μ -([³H]DAMGO, 0.02–15 nM, 45 min, 35 °C), the δ -([³H][Ile^{5,6}]deltorphin II, 0.05–12 nM, 45 min, 35 °C) or the κ -([³H]U69,593, 0.02–20 nM, 30 min, 30 °C) selective opioid radioligands. Reactions were terminated by rapid filtration through Whatman GF/B pretreated with 0.1%

polyethyleneimine ([³H]U69,593) or GF/C ([³H]DAMGO and [³H][Ile^{5,6}]deltorphin II) glass fibre filters using a Brandel Cell Harvester, followed by three washings with 5 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The bound radioactivity was measured in Ultima Gold scintillation cocktail, using a Beckman LS1701 liquid scintillation counter. Non-specific binding was determined in the presence of 10 μ M unlabeled naloxone. All experiments were carried out

Table 2
Influence of chronic arthritic pain on levels of immunoreactive dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ in tissues from control and adjuvant arthritic rats

Tissue	Dynorphin B (fmol/mg)	Percentage increase in dynorphin B ^a (%)	[Met ⁵]enkephalin-Arg ⁶ -Phe ⁷ (fmol/mg)	Percentage increase in [Met ⁵]enkephalin-Arg ⁶ -Phe ⁷ (%)
<i>STR</i>				
C	5.58 ± 0.29 (10)	64	12.62 ± 1.72 (11)	182
A	9.13 ± 0.67 (10) ^b		35.6 ± 6.7 (10) ^c	
<i>HT</i>				
C	4.23 ± 0.48 (12)	113	6.65 ± 0.82 (12)	190
A	8.99 ± 0.98 (12) ^b		19.31 ± 2.61 (12) ^b	
<i>HPC</i>				
C	4.99 ± 0.40 (12)	29	1.75 ± 0.15 (12)	102
A	6.46 ± 0.68 (12)		3.54 ± 0.57 (12) ^c	
<i>FCX</i>				
C	9.54 ± 0.75 (12)	187	6.62 ± 0.56 (12)	158
A	27.4 ± 2.0 (7) ^b		17.1 ± 2.5 (10) ^b	
<i>OCX</i>				
C	7.45 ± 0.72 (12)	121	5.08 ± 0.55 (12)	144
A	16.5 ± 1.2 (12) ^b		12.4 ± 1.2 (12) ^b	
<i>AL</i>				
C	4.31 ± 0.67 (10)	– 45	0.56 ± 0.12 (12)	– 18
A	2.35 ± 0.39 (11) ^d		0.46 ± 0.08 (11)	
<i>NIL</i>				
C	648 ± 55 (12)	– 76	3.78 ± 0.94 (12)	– 49
A	157 ± 28 (10) ^c		1.91 ± 0.27 (12)	
<i>LSC</i>				
C	1.91 ± 0.19 (12)	232	6.69 ± 0.90 (12)	160
A	6.34 ± 0.47 (12) ^b		17.4 ± 1.4 (12) ^b	

C=control non-arthritic rats; A=arthritic rats; STR=striatum; HT=hypothalamus; HPC=hippocampus; FCX=frontal cortex; OCX=occipital cortex; AL=anterior lobe; NIL=neurointermediate lobe; LSC=lumbar spinal cord.

Values represent mean ± S.E.M.; *n* is in parentheses.

^a Data are expressed as percent (%) increase in dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ levels in adjuvant arthritic rats, respectively, as compared to controls.

^b Significance of arthritic vs. control animals: $P < 0.001$ (Student's two-tailed *t*-test).

^c Significance of arthritic vs. control animals: $P < 0.01$ (Student's two-tailed *t*-test).

^d Significance of arthritic vs. control animals: $P < 0.05$ (Student's two-tailed *t*-test).

in duplicate. Protein concentration was measured by the Lowry method (Lowry et al., 1951).

2.4. Data analysis

Results are expressed as the mean \pm S.E.M. The peptide levels in different brain structures and spinal cord, expressed as fmol/mg wet weight tissue, were calculated using the Statview 4.5 program. Rosenthal analysis was performed by first-order non-linear regression analysis of the data from saturation isotherms (Rosenthal, 1967). Binding parameters, represented by equilibrium dissociation constant (K_d , in nM) and receptor density (B_{max} , in fmol/mg protein), were calculated from these plots using the Slide Write program. Statistical significance was calculated with Student's *t*-test. A *P* value <0.05 was considered statistically significant.

3. Results

3.1. Parameters of inflammation

In agreement with the previous observations (Ahmed et al., 1995a; Spetea et al., 1999), rats developed signs of inflammation 9–12 days after adjuvant injection. There was

bilateral hind paw swelling, warmth and redness. Radiology (not shown) of the ankle joints, performed on day 29, showed bony erosions, periosteal thickening and joint space narrowing. Histology (not shown) disclosed synovial hypertrophy and chronic inflammatory cell infiltration. Control animals showed no macroscopical, histological or radiological signs of inflammation. There was a significant reduction in body weight of arthritic rats in contrast to control animals. Comparison of ankle joint diameter showed a significant increase in arthritic rats (50%, $P<0.001$) compared to control rats. Chronic arthritis also induced a significant increase in spleen weight with a corresponding decrease in thymus weight (Table 1).

3.2. Changes in opioid peptide levels

In Table 2, the levels of immunoreactive dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ in various brain areas, pituitary and lumbar spinal cord as determined by radioimmunoassay are shown. Levels of opioids are expressed as fmol/mg tissue. Changes in levels of opioids in arthritic rats are also presented as a percentage (%) change compared to controls.

At 4 weeks post-inoculation, increased levels of dynorphin B were observed in the striatum, hypothalamus, frontal and occipital cortex and in the lumbar region of the spinal

Table 3

Properties of opioid binding sites as determined by Rosenthal analysis in tissues from control and adjuvant arthritic rats

Tissue	³ H]DAMGO (μ -sites)		³ H][Ile ^{5,6}]deltorphin II (δ -sites)		³ H]U69,593 (κ -sites)	
	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)
STR						
C	1.6 \pm 0.4 (5)	44.8 \pm 3.4 (5)	2.7 \pm 0.6 (3)	67.6 \pm 1.2 (3)	N.D.	N.D.
A	1.9 \pm 0.6 (3)	51.8 \pm 3.4 (3)	2.1 \pm 0.1 (3)	65.8 \pm 6.8 (3)	N.D.	N.D.
HT						
C	2.7 \pm 0.8 (2)	35.5 \pm 0.3 (2)	N.D.	N.D.	3.4 \pm 0.1 (2)	8.3 \pm 1.6 (2)
A	2.3 \pm 0.1 (2)	47.1 \pm 3.4 (2)	N.D.	N.D.	3.1 \pm 0.2 (2)	11.4 \pm 0.1 (2)
HPC						
C	1.3 \pm 0.4 (3)	77.0 \pm 5.1 (3)	3.9 \pm 0.1 (2)	36.3 \pm 7.2 (3)	N.D.	N.D.
A	1.6 \pm 0.3 (5)	54.6 \pm 6.7 (4)	3.5 \pm 0.6 (3)	22.2 \pm 1.4 (3)	N.D.	N.D.
FCX						
C	1.3 \pm 0.2 (7)	69.8 \pm 8.1 (7)	2.8 \pm 0.3 (6)	87.8 \pm 2.9 (6)	2.8 \pm 0.6 (5)	11.5 \pm 1.4 (5)
A	1.2 \pm 0.3 (6)	90.7 \pm 6.7 (5)	2.8 \pm 0.5 (5)	132 \pm 12 (5) ^a	2.9 \pm 0.4 (6)	10.7 \pm 0.6 (6)
OCX						
C	0.7 \pm 0.1 (8)	68.5 \pm 4.3 (8)	2.8 \pm 0.2 (8)	95.2 \pm 10.3 (8)	1.9 \pm 0.3 (4)	7.9 \pm 1.4 (4)
A	2.6 \pm 0.8 (7) ^b	56.8 \pm 7.0 (7)	2.6 \pm 0.2 (6)	94.8 \pm 5.0 (6)	1.6 \pm 0.2 (5)	8.5 \pm 0.5 (5)
LSC						
C	1.6 \pm 0.5 (4)	37.3 \pm 2.6 (4)	2.5 \pm 0.9 (4)	11.8 \pm 2.2 (4)	N.D.	N.D.
A	1.6 \pm 0.2 (4)	36.6 \pm 6.1 (4)	1.9 \pm 0.8 (4)	10.1 \pm 1.9 (4)	N.D.	N.D.

C = control non-arthritic rats; A = arthritic rats; STR = striatum; HT = hypothalamus; HPC = hippocampus; FCX = frontal cortex; OCX = occipital cortex; LSC = lumbar spinal cord; N.D. = not detectable.

Values represent mean \pm S.E.M.; *n* is in parentheses. For each experiment, tissues were pooled from two to three rats.

^a Significance of arthritic vs. control animals: $P<0.01$ (Student's two-tailed *t*-test).

^b Significance of arthritic vs. control animals: $P<0.05$ (Student's two-tailed *t*-test).

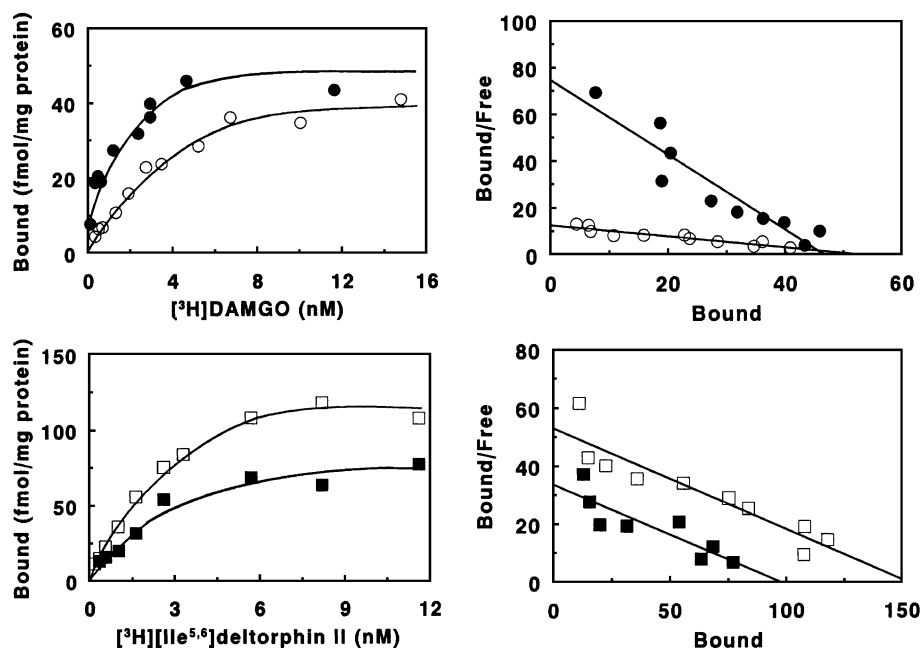


Fig. 1. Representative saturation isotherms for binding of $[^3\text{H}]\text{DAMGO}$ to occipital cortex membranes (upper panels) and $[^3\text{H}][\text{Ile}^{5,6}]\text{deltorphin II}$ to frontal cortex membranes (lower panels) from control (filled symbols) and arthritic (open symbols) rats. Membranes were incubated with increased concentrations of appropriate opioid radioligand in the absence or in the presence of 10 μM naloxone for 45 min at 35 $^{\circ}\text{C}$. Rosenthal transformation of the saturation data is presented in the right panels.

cord of rats with inflammation compared to controls (Table 2). Some increases, although not statistically significant, were also detected in the hippocampus of arthritic animals. The most pronounced increase (232%) was found in the lumbar spinal cord of arthritic rats.

Tissue levels of immunoreactive $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$ were also significantly elevated due to chronic inflammation (Table 2). Moreover, the rank order of $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$ levels in different brain regions was somewhat similar to dynorphin B. In the lumbar cord, the rise in dynorphin B was greater than that for $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$, 232% vs. 160%, respectively. Striatal $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$ levels were also elevated in arthritic rats by 184%, while a lower increase (64%) was observed for dynorphin B. A significant depletion in dynorphin B concentration was seen in the anterior and neuro-intermediate lobes of the pituitary gland. In contrast to dynorphin B, levels of $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$ were not significantly altered in any of these regions. Notably, the measured concentrations of $[\text{Met}^5]\text{enkephalin-Arg}^6\text{-Phe}^7$ in the anterior and neurointermediate lobes were very low, close to the detection limit in the radioimmunoassay. Overall, a very good correlation was observed between the changes of the two opioid peptide systems across all analysed structures.

3.3. Changes in opioid receptor binding

In Table 3, quantitative differences in μ -, δ - and κ -opioid receptors activity in inflamed vs. non-inflamed animals as determined by receptor binding assay are shown. The

changes in affinity (K_d) and binding density (B_{max}) of selective μ - ($[^3\text{H}]\text{DAMGO}$), δ - ($[^3\text{H}][\text{Ile}^{5,6}]\text{deltorphin II}$) and κ - ($[^3\text{H}]\text{U69,593}$) opioid radioligands were assessed by saturation binding experiments. All saturation curves were best fitted a one-site model for each receptor type.

Equilibrium binding parameters for the opioid radioligands, as determined by Rosenthal analysis, did not significantly differ between tissues from non-arthritic and arthritic rats. The calculated binding affinities (K_d) were similar in different tissues. There were no differences between control and polyarthritic animals with regard to binding affinities of the type-selective opioid radioligands. Only in the occipital cortex, a decrease in the affinity at the μ -opioid binding site was noted (Table 3 and Fig. 1). μ -Opioid radioligand binding in arthritic rats was elevated above control levels, although not statistically significant, in the striatum, hypothalamus and frontal cortex, while a decrease was detected in the hippocampus and occipital cortex. No change in δ -binding sites was observed in the striatum, hippocampus and occipital cortex, but an increase in B_{max} was noted in the frontal cortex of arthritic rats (Table 3 and Fig. 1). No alterations in the κ -opioid radioligand binding were observed in the frontal and occipital cortexes, but a slight increase was found in the hypothalamus of arthritic rats (Table 3).

4. Discussion

Changes of the endogenous opioid systems, implicated in the central transmission of nociceptive information, were

assessed in rats with chronic polyarthritis. The present study shows that in inflammatory pain conditions, pronounced alterations in the cerebral and spinal pro-dynorphin and pro-enkephalin systems and in multiple opioid receptors occur.

This work involved spinal cord and areas of the brain that are considered to have a direct role in pain processing, or are associated with the rostral limbic system or associated cortical areas. Our findings support the view that chronic arthritis does not induce changes in opioid receptors. In addition, influences on discrete pools of particular opioid peptides are apparent as earlier reported (Millan et al., 1986, 1988). These observations reflect alterations in the functional activity of multiple opioid systems that are known to be implicated in the control of nociception and inflammation.

The present study focused on two opioid peptide systems, pro-dynorphin and pro-enkephalin. The endogenous ligands investigated were dynorphin B, which derives exclusively from pro-dynorphin, and [Met⁵]enkephalin-Arg⁶-Phe⁷, which is a pro-enkephalin-derived heptapeptide. Dynorphin B acts primarily on the κ -opioid receptor while [Met⁵]enkephalin-Arg⁶-Phe⁷ has been described to be a selective ligand for the κ_2 -opioid subtype (Benyhe et al., 1997). To our knowledge, no data are available concerning the contribution of [Met⁵]enkephalin-Arg⁶-Phe⁷ to chronic inflammatory pain.

We report in this study that levels of dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ were significantly elevated in chronic arthritis in the striatum, hypothalamus, frontal and occipital cortexes and lumbar region of the spinal cord. Immunoreactive dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ levels were increased by 232% and 160%, respectively, in the lumbar cord of arthritic compared to non-arthritic animals. It is notable that across all regions, the changes of the two peptide systems were very well correlated, thus suggesting that the dynorphin and enkephalin systems are acting in parallel in response to inflammation and nociception. The pronounced elevation of dynorphin B is consistent with other reports on the increase in this neuropeptide, as well as for other endogenous opioids (i.e. [Met⁵]enkephalin, α -neo-endorphin) (Cesselin et al., 1980; Millan et al., 1986, 1987).

Arthritic pain was also accompanied by a decrease in the content of immunoreactive dynorphin B in both anterior and neurointermediate lobes of the pituitary gland, while levels of [Met⁵]enkephalin-Arg⁶-Phe⁷ were not elevated in any of these regions. Previous studies have given contradictory results regarding the effect of chronic arthritic pain on pituitary dynorphin, when an increase in this peptide was reported in the anterior lobe (Millan et al., 1986). The functional significance of pituitary dynorphin B and [Met⁵]enkephalin-Arg⁶-Phe⁷ remains to be elucidated, although it is known that this region represents a rich source of opioid peptides and various stimuli can cause the release of pituitary opioids (Guillemin et al., 1977; Millan et al., 1984, 1985, 1986). The depletion in pituitary opioids in arthritic rats, observed in the present study, reflects a release of these peptides into the periphery. The changes in this region were

very marked when compared to those detected in other brain areas. In contrast to pituitary, in the lumbar spinal cord and in the other brain structures, an elevation in the opioid peptides was noted. Possibly, this rise reflects an enhancement of opioid biosynthesis during chronic arthritic pain. These findings suggest that long-term exposure to noxious signals, as in inflammatory or chronic pain conditions, not only stimulate the synthesis of opioid peptides but also their release into the periphery to the site of tissue damage where they could act to reduce pain (Millan et al., 1987; Stein et al., 1990a,b; Mousa et al., 2001).

In the present study, saturation binding experiments using selective μ -, δ - or κ -opioid radioligands failed to demonstrate changes in arthritic rats compared to the controls in the number of opioid binding sites or in the affinity in the analysed tissues. Previously, Millan et al. (1986), using [³H]diprenorphine, have also reported that in chronic arthritic rats, no significant changes in opioid receptors occur in lumbosacral spinal cord, thalamus and midbrain.

According to our results, only in the frontal cortex, a relative increase in the number of δ -, and in the occipital cortex a decrease in affinity to μ -opioid receptors, was seen in chronic inflammation. Moreover, both μ - and δ -opioid receptors showed to be up-regulated in the frontal cortex (<30%), which could imply an enhancement of sensory integration due to inflammation. Our findings of low δ -opioid binding in the hypothalamus and low κ -opioid binding in the striatum and hippocampus are consistent with the other reports (Mansour et al., 1988). The spinal cord, which is known to be involved in processing nociceptive information, contains large numbers of μ -binding sites, while there are intermediate numbers of δ -sites. There is evidence that there are very few κ -opioid binding sites in the rat spinal cord compared to the total population of opioid receptors (Stevens et al., 1991; Besse et al., 1992). In this study, no κ -opioid receptors were detected in spinal cord, probably due to the fact that a binding assay, rather than the autoradiographical approach has been employed to assess the quantitative levels of κ -opioid receptors in the spinal cord. On the other hand, in a work of Wood et al. (1989), using radioligands with high affinity for κ -opioid binding sites (e.g. [³H]U69,598, and [³H]dynorphin A(1–9)) and employing receptor binding assay in homogenates of lumbosacral spinal cord from the rat, low levels of binding were obtained.

In an early report of Millan et al. (1986), a relative reduction in the proportion of κ -, compared to μ -opioid receptors, was seen in the lumbosacral cord of polyarthritic rats, while levels of opioid peptides (e.g. dynorphin) were found to be elevated. It was described in enkephalin knockout rats that there is an up-regulation of the target receptor in the brain to compensate the loss of the peptide (Brady et al., 1999). In our study, the increase in the activity of an endogenous ligand (dynorphin B) due to chronic inflammation does not lead to a change in opioid receptor (κ) expression with which it is presumed to interact, thus suggesting that a dynamic interplay between endogenous ligand levels and receptor levels occurs.

Our findings on chronic arthritis agree with the previous studies on animals and humans, where no central changes in opioid receptors were demonstrated, while alterations in opioid peptide expression have been reported (Cesselin et al., 1980; Millan et al., 1986; Jones et al., 1994). The mechanism underlying these observations remains unclear. Moreover, up to date, there is no explanation available for the fact that opioid binding sites are altered during acute but not chronic stages of inflammation. In a recent study of Danziger et al. (2001), changes of tonic modulation acting on the spinal transmission of nociceptive information were described in rats with acute and chronic monoarthritis. Tonic descending inhibition of convergent neurones with input from the inflamed ankle was enhanced during the acute stage, while a decrease was observed to occur during the chronic stage of monoarthritis. After the induction of peripheral inflammation, the axonal transport of opioid receptors in fibres of the sciatic nerve was reported to be greatly enhanced, which leads to receptor up-regulation on peripheral nerve terminals (Hassan et al., 1993; Mousa et al., 2001).

Accumulating evidence from both clinical and experimental studies indicate that both central and peripheral nervous systems are involved in the pathogenesis and development of arthritis (Stein, 1995; Walker et al., 1997). The contribution of the nervous system to inflammation is supported by studies showing that chemical (capsaicine pretreatment) or surgical denervation decreases the severity of inflammation (Ahmed et al., 1995a,b; Spetea et al., 1999). In the present study, we found that a network of cortical regions is reliably activated by chronic inflammatory pain. These findings are consistent with the hypothesis that substantial increases occur in the levels of central opioid peptides and in occupancy of their receptors by endogenous opioids during chronic inflammatory pain. A key observation is that opioid peptides and opioid receptors are known to be implicated in afferent and efferent central transmission of nociceptive information from and to the peripheral tissues via the spinal cord. During both acute and chronic stages of inflammation, descending controls tend to dampen central changes associated with inflammation (Danziger et al., 1999, 2001). Thus, inflammation apparently induces a unique simultaneous up-regulation of peripheral opioid receptors and of their endogenous ligands (Stein et al., 1988, 1990b; Hassan et al., 1993; Mousa et al., 2001). This is in contrast to the situation in the central nervous system where an up-regulation of endogenous ligands is typically paralleled by no alterations, as found in this study, or a down-regulation of receptors as reported by others (Morris, 1993). It was proposed that functional plasticity might play a significant role in the pathophysiology of chronic pain syndromes and an imbalance of descending controls could exacerbate the consequences of the central changes induced by inflammatory processes, and thus, result in increased and persistent pain (Danziger et al., 2001).

In conclusion, the present study shows that in chronic arthritis, there is an enhancement of the functional activity

of spinal and supraspinal opioidergic neurones. These results indicate that opioid receptor changes do not follow the peptide alterations of pro-dynorphin and pro-enkephalin systems. Thus, dynamic modification and modulation of nociceptive information takes place during chronic inflammation. This supports the key role of the central nervous system in chronic inflammatory pain conditions.

Acknowledgements

This study was supported by grants from the European Community (EPLIA, QLK6-1999-02334) and from the Swedish Medical Research Council (12X-08652-09B; 14X-12588-04B). Mariana Spetea was supported by the fellowships from the Swedish Institute and Wener-Gren Foundation.

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