Loss of vasomotor responsiveness to the μ-opioid receptor ligand endomorphin-1 in adjuvant monoarthritic rat knee joints

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McDougall, Jason J., A. Kursat Barin, and Chelsea M. McDougall. Loss of vasomotor responsiveness to the μ-opioid receptor ligand endomorphin-1 in adjuvant monoarthritic rat knee joints. Am J Physiol Regul Integr Comp Physiol 286: R634–R641, 2004.—Endomorphin-1 is a short-chain neuropeptide with a high affinity for the μ-opioid receptor and has recently been localized in acutely inflamed knee joints where it was found to reduce inflammation. The present study examined the propensity of endomorphin-1 to modulate synovial blood flow in normal and adjuvant-inflamed rat knee joints. Under deep urethane anesthesia, endomorphin-1 was topically applied to exposed normal and 1 wk adjuvant monoarthritic knee joints (0.1 ml bolus; 10⁻¹²–10⁻⁹ mol). Relative changes in articular blood flow were measured by laser Doppler perfusion imaging and vascular resistances in response to the opioid were calculated. In normal knees, endomorphin-1 caused a dose-dependent increase in synovial vascular resistance and this effect was significantly inhibited by the specific μ-opioid receptor antagonist t-Phe-Cys-Tyr-t-Trp-Org-3177-OH (P < 0.0001, 2-factor ANOVA, n = 5–7). One week after adjuvant inflammation, the hypoaemic effect of endomorphin-1 was completely abolished (P > 0.0001, 2-factor ANOVA, n = 5–7). Immunohistochemical analysis of normal and adjuvant-inflamed joints showed a ninefold increase in endomorphin-1 levels in the monoarthritic knee compared with normal control. Western blotting and immunohistochemistry revealed a moderate number of μ-opioid receptors in normal knees; however, μ-opioid receptors were almost undetectable in arthritic joints. These findings demonstrate that peripheral administration of endomorphin-1 reduces knee joint blood flow and this effect is not sustainable during advanced inflammation. The loss of this hypoaemic response appears to be due to downregulation of μ-opioid receptors as a consequence of endomorphin-1 accumulation within the arthritic joint.

arthritis; blood flow; opioids; neuropeptides; inflammation

IT HAS BEEN KNOWN for some time that the peripheral nervous system significantly contributes to the development and progression of chronic joint diseases such as rheumatoid arthritis. Supportive evidence of this phenomenon comes from numerous studies that have shown that surgical or chemical sensory denervation of joints considerably impedes the development of experimentally induced arthritis (3, 7, 19, 20). The mechanisms responsible for this neurogenic component of inflammatory joint disease are still unclear but appear to involve the release of proinflammatory agents into the joint that then act on the synovial vasculature to cause vasodilatation and protein extravasation. Experimentally, it has been shown that antinociceptive effects. The endogenous ligand for the μ-opioid receptor, has been shown to ameliorate the clinical signs of experimentally induced arthritis (20, 33), whereas δ- and κ-opioid agonists are also capable of reducing the morphological and pathophysiological changes associated with joint inflammation (4, 10, 29, 34, 35). The precise role of the ORL-1 receptor ligand nociceptin in joint inflammation is less clear as the peptide has been found to produce dual vasomotor effects in rat synovium (24). Although the opioid family appears to be promising therapeutic targets for the treatment of inflammatory joint disease, chronic opioid use is also renowned for generating harmful side effects as well as being associated with drug tolerance and possible addiction. An alternative therapy to synthetic opioids may be the use of endogenous opioid peptides that, due to their natural occurrence in the body, may be less inclined to impart unwanted side effects. The endogenous ligand for the μ-opioid receptor has recently been identified as endomorphin, of which there are two types: endomorphin-1 and endomorphin-2 (11, 37). Originally localized in the central nervous system (8, 11, 22, 31, 37), evidence is accumulating to suggest that endomorphin may also function peripherally. Khalil et al. (16) found that local administration of endomorphin-1 to a skin blister model inhibited the vascular response to substance P and afferent C-fiber stimulation at the site of inflammation (16). Immunohistochemical and radioimmunoassay techniques have detected endomorphin in rat joints (13, 25), and peripheral application of endomorphin-1 caused a reduction in synovial blood flow by inhibiting the release of vasodilator neuromediators from capsaicin-sensitive nerves (2).
This study sought to examine whether endomorphin-1 could impart an anti-inflammatory effect in rat knee joints by using a model of inflammatory arthritis. To this end, the vasomodulatory effects of endomorphin-1 were assessed in normal and adjuvant monoarthritisic knees. Additional immunohistochemical experiments were also carried out to try to localize endomorphin-1 and \( \mu \)-opioid receptors in knees as well as determining whether adjuvant inflammation alters the distribution of the peptide and the receptor to which it binds.

**MATERIALS AND METHODS**

**Animals and surgical procedures.** Thirty-four outbred male Wistar rats (291–400 g; University of Calgary colony) were housed in cages at room temperature under a 12:12-h (7 AM-7 PM) light-dark cycle, and rodent food and tap water were available ad libitum. The animal handling and surgical procedures employed in this study adhered to the guidelines set out by the Canadian Council for Animal Care.

The animals were deeply anesthetized with urethane (25% stock solution, 2 g/kg ip), and the depth of surgical anesthesia was confirmed by the absence of the hindpaw withdrawal reflex. The trachea was cannulated to permit unrestricted airflow, and in the case of respiratory difficulties the animal was artificially ventilated with 100% oxygen by a Harvard rodent ventilator (Harvard Apparatus). Next, the left carotid artery was cannulated with a heparin-saline-filled cannula (Portex Fine Bore Tubing, 0.40 mm internal diameter, 0.80 mm outer diameter; SIMS Portex) that was attached to a pressure transducer to permit continuous blood pressure monitoring (Pressure Monitor BP-1, World Precision Instruments). To obtain unobstructed knee joint perfusion measurements, the skin and overlying fascia of the anteromedial portion of the knee was removed with the aid of a binocular dissection microscope. Finally, the animals were positioned supine on an electric heated blanket (TR-100, Fine Science Tools) to maintain their internal body temperatures at 37°C as measured by a rectally inserted electronic thermometer. The exposed knee joints were frequently washed with warm (37°C) physiological saline (0.9% NaCl) to prevent tissue dehydration.

**Adjuvant inflammation model.** Unilateral inflammation was induced by an intra-articular injection of 0.2 ml Freund’s complete adjuvant (Sigma Chemical) 0.1 ml into the posterior region of the knee joint and 0.1 ml into the anterior compartment 1 wk before the blood flow experiments. Swelling was present in all treated knee joints, and a comparison of the knee joint widths before and 1 wk after induction of inflammation confirmed an inflammatory reaction to the adjuvant (a mean increase of 18.2%, \( P = 0.0014 \), paired 2-tailed Student’s t-test; \( n = 5 \)).

**Knee joint perfusion and vascular resistance measurements.** The effects of endomorphin-1 on knee joint perfusion were measured with a laser Doppler perfusion Imager (LDI; Moor Instruments). This noninvasive technique generates a two-dimensional representation of tissue perfusion that is based on the concentration and speed of circulating erythrocytes in the articular microvasculature. All perfusion images were analyzed by LDI processing software (Moor Instruments) and the average flux for the anteromedial aspect of the joint calculated and expressed in arbitrary perfusion units (PU). Concurrent with each perfusion scan, the mean arterial blood pressure (MAP) of the animal was recorded and used to calculate the vascular resistance (MAP divided by blood flow) of the synovial blood vessels.

**Endomorphin-1 administration.** Endomorphin-1 (Calbiochem) was applied topically to the exposed rat knee joint as a 0.1 ml bolus (37°C) over a dose range of 10\(^{-12}\)–10\(^{-9}\) mol. This dose range was chosen as it falls within the optimal region of the previously described endomorphin-1 dose-response curve (2). An initial (basal) perfusion measurement of the knee was recorded and, after administration of the opioid, perfusion scans were taken for each dose at 0, 2, 4, 6, 8, and 10 min. The dose sequencing was randomized to prevent possible tachyphylactic effects. Between doses of endomorphin-1, the knee joints were topically washed with warmed (37°C) saline (0.9% NaCl) to eliminate residual endomorphin-1 and prevent tissue dehydration.

In other experiments, the specific \( \mu \)-opioid receptor antagonist \( \text{D-Phe-Cys-Tyr-\text{d-Trp-Orn-Thr-Pen-Thr}} \) amide (CTOP) was used to verify that the hypoemic effect of endomorphin-1 is mediated by this receptor. In six normal exposed knee joints, warmed (37°C) 0.1 ml bolus aliquots of 10\(^{-8}\) mol CTOP (Sigma Chemical) were administered before and in conjunction with endomorphin-1 over the randomized dose range of 10\(^{-12}\)–10\(^{-9}\) mol and synovial blood flow was measured as described above.

**Histology and immunohistochemistry.** Eleven rats were used for histological and immunohistochemical assessment of which six rats underwent unilateral inflammation as described above (1 wk recovery) and five rats served as normal controls. On the day of tissue harvest, animals were urethane anesthetized (2 g/kg ip) and the saphenous artery was cannulated proximal to the medial articular artery. The animal was placed in dorsal recumbency on a bed of ice and the knee joint was initially flushed with 3 ml of heparinized saline (0.9% NaCl; 4°C) using a syringe pump (model 210, KD Scientific, New Hope, PA). Subsequently, the knee was perfused with 3 ml of 4% paraformaldehyde with one-half of the fixative given while the animal was alive and the remaining 1.5 ml administered after animal death. The perfused hindlimbs were removed and immersed in 4% paraformaldehyde for 2 days. After this time, the muscles were removed and the hindlimb was placed in a decalcifying solution (Cal-Ex, Fisher Scientific, Ontario, Canada) for 3–5 days. The tissues were then cryoprotected in 30% sucrose in PBS for 2 days and the joint embedded in OCT mounting media (Fisher Scientific) before being frozen in 2-methylbutane and stored at −20°C until sectioning. Twelve-micrometer-thick sections were cut on a cryostat and mounted onto Superfrost Plus slides before drying at 36°C for 7 days. Trichromatic staining was performed on every fifth section to visualize the anatomical structures in the joint, thereby assisting the immunolocalization studies. Briefly, the sections were rinsed three times with double distilled water, then sequentially immersed in solutions of hematoxylin for 15 min (Gills #3), then 0.05% fast green for 6 s, and finally in 0.1% safranin O for 2 min. The slides were then cleared with alcohol (consecutively increasing concentrations of 80–100%) and finally 100% xylene. Sections were placed under a coverslip and viewed under bright light microscopy.

Immunohistochemical staining of the sections for either endomorphin-1 or for the \( \mu \)-opioid receptor was carried out. Initially, slides were rinsed with 1 M PBS (pH 7.4) containing 0.4% Triton X-100 (Sigma-Aldrich Canada). The slides were then incubated with 10% normal goat blocking serum at room temperature for 2 h, then incubated with a primary antibody cross-reactive to either endomorphin-1 (rabbit anti-rat polyclonal endomorphin-1, 1:75, Phoenix Pharmaceuticals, Belmont, CA) or the \( \mu \)-opioid receptor (rabbit anti-rat polyclonal, 1:1,000 Oncogene Research Products, Cambridge, MA) in a humidity chamber at 4°C for 48 h. Slides were then rinsed in 1 M PBS (5 × 10 min). After a second block with 10% normal goat serum, the sections were incubated with a secondary antibody at room temperature for 2 h, then incubated with a primary antibody cross-reactive to either endomorphin-1 (rabbit anti-rat polyclonal endomorphin-1, 1:75, Phoenix Pharmaceuticals, Belmont, CA) or the \( \mu \)-opioid receptor (rabbit anti-rat polyclonal, 1:1,000 Oncogene Research Products, Cambridge, MA) in a humidity chamber at 4°C for 48 h. Secondary antibody at room temperature for 2 h, then incubated with a secondary antibody at room temperature for 2 h, then incubated with a primary antibody cross-reactive to either endomorphin-1 (rabbit anti-rat polyclonal endomorphin-1, 1:75, Phoenix Pharmaceuticals, Belmont, CA) or the \( \mu \)-opioid receptor (rabbit anti-rat polyclonal, 1:1,000 Oncogene Research Products, Cambridge, MA) in a humidity chamber at 4°C for 48 h. Slides were then rinsed in 1 M PBS (5 × 10 min). After a second block.

Against the primary antibody: 1) immunohistochemistry was performed but with the omission of the primary antibody or the biotinylated secondary antibody, and 2) preadsorption controls were assessed in which the endomorphin-1 primary antibody was preincubated with 10 µM endo-
Morphin-1 for 24 h at 4°C. No immunoﬂuorescence was detected in any of these control experiments. Confirmation of primary antibody viability was demonstrated by positive binding in sections of rat brain and dorsal root ganglia as previously reported (1, 21).

**Semi-quantitative analysis of endomorphin-1 immunoreactivity.** Representative sections of normal and inﬂamed synovium were used for semi-quantitative analysis of endomorphin-1 immunoreactivity levels.

Multiple sections from medial, lateral, anterior, and posterior regions of the joint were chosen to include every aspect of the knee. Under ×100 magniﬁcation, microscopic images were taken with a constant integration time using a Zeiss Axiocam (Carl Zeiss Vision, München, Germany) connected to a Zeiss Axiostar 2 Plus ﬂuorescent microscope (Carl Zeiss, Jena, Germany). Up to 200 overlapping images of each synovial section were acquired and these images were “stitched” together using Adobe

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Photoshop Elements software (Adobe Systems, San Jose, CA) to form a mosaic image of the synovium. This composite image was then analyzed using ImageJ software (download site http://rsb.info.nih.gov/ij). First, the image was converted to 8-bit and then thresholded to remove background fluorescence, thereby leaving only positively stained nerve fibers that were intensely fluorescing above the defined threshold level. A region of interest was carefully circumscribed around the synovium and the area occupied by endomorphin-1-containing immunopositive nerve fibers was calculated. Results were expressed as the percent area fraction of positively stained nerve fibers compared with the total synovial area. Eight composite images were analyzed for normal knees, and thirteen images from adjuvant-inflamed joints were assessed.

Western blot analysis. Comparisons of μ-opioid receptor levels between normal and adjuvant-inflamed knees was validated by Western blot analysis. Joint capsules were excised from normal and adjuvant-inflamed knees and flash frozen to −80°C. Tissues were dismembranated and reconstituted in homogenization buffer [50 mM PBS, 70 mM sucrose, 1 mM EDTA, 5% β-mercaptoethanol, protease inhibitor cocktail (Complete Mini Tablets, Roche Diagnostics, Mannheim, Germany)]. Samples were then centrifuged (14,000 rpm, 20 min) and the supernatant (5 μg protein) was electrophoresed on a 4–20% gradient SDS-polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane by electrophoretic blotting for 90 min at 200 mA. The blot was then washed twice in PBS-Tween (PBS containing 0.1% Tween 20), and nonspecific binding sites were blocked with 2% fat-free milk for 2 h at room temperature. Blots were incubated with the μ-opioid receptor primary antibody (1:10,000 dilution) for 48 h at 4°C. Membranes were again washed in PBS-Tween (4 × 5 min) incubated with a secondary goat anti-rabbit IgG antibody conjugated to horseradish peroxidase. After a final wash, bound antibodies were detected with an enhanced chemiluminescence kit (Amersham International, Amersham, Buckinghamshire, UK) and recorded by digital photography.

Statistical analysis. Statistical analyses of the results were performed with GraphPad Prism software (GraphPad Software). Data sets were found to be normally distributed and, therefore, analyzed using parametric statistical tests. ANOVA was used to examine differences in perfusion changes within (1-way ANOVA) and between (2-way ANOVA) different groups. Student’s t-tests were used to compare CTOP effects on basal resistance as well as immunoreactivity levels between normal and inflamed synovium. A significance level of $P < 0.05$ was used for each test and all reported values represent means ± SE.

RESULTS

Immunohistochemical analysis. Immunopositive staining for endomorphin-1 was found in both the anterior and posterior compartments of normal and adjuvant-inflamed rat knee joint capsules. Endomorphin-1 containing nerves were not only found coursing throughout the tissue matrix of the capsule, but were also localized in close proximity to synovial blood vessels to form discrete neurovascular bundles (Fig. 1). Semiquantitative analysis of immunohistochemically stained sections (Fig. 2) revealed that the amount of endomorphin-1 in adjuvant-inflamed synovium was about nine times higher than in normal knee joints ($P < 0.001$, 2-tailed unpaired Student’s $t$-test; $n = 8$ and 13 for normal and adjuvant-inflamed knee joints respectively).

Fluorescent staining of μ-opioid receptors was clearly identifiable on articular nerves located in normal rat synovium (Fig. 3). Similar to endomorphin-1 containing nerves, the opioid receptors ramified throughout the tissue matrix and were occasionally found on nerves apposing synovial blood vessels. In contrast, μ-opioid receptors were rarely seen in adjuvant-inflamed knee joints with an absence of immunopositive staining in almost all sections. To corroborate this observation, Western blot analysis of normal and adjuvant-treated knees was carried out. In control knees a discrete band at ~50 kDa was observed (which is consistent with the known molecular weight of μ-opioid receptors), whereas in 1 wk adjuvant-inflamed joints μ-opioid receptor chemiluminescence was undetectable (Fig. 4).

Effect of endomorphin-1 on knee joint blood flow. Topical administration of endomorphin-1 to exposed normal knee joints resulted in a consistent fall in synovial blood flow across the dose range tested. The actual changes in blood flow from control were 792.2 ± 121 to 569.1 ± 78 PU (10$^{-12}$ mol), 601.4 ± 64 to 429.4 ± 55 PU (10$^{-11}$ mol), 745.1 ± 89 to 563.7 ± 88 PU (10$^{-10}$ mol), and 636.7 ± 71 to 454.2 ± 87 PU (10$^{-9}$ mol). To obviate any influence from a fluctuation in systemic blood pressure, joint vascular resistance was calculated and found to increase after endomorphin-1 administration with the maximal effect tending to occur 6 min after administration (Fig. 5). Joint perfusion subsequently returned back to control levels by 10 min. The maximal hypoaemic effect of endomorphin-1 occurred with the highest dose of the drug with vascular resistance increasing by 55.8 ± 17.5% ($n = 7$) compared with control (Fig. 6A). It should be noted that the vascular resistance measured before each dose of drug was not significantly different from initial basal resistance levels ($P = 0.71$, repeated-measures 1-way ANOVA), indicating complete recovery of joint perfusion between doses of endomorphin-1. Coadministration of the μ-opioid receptor antagonist CTOP (10$^{-5}$ mol) completely abolished the hypoaemic effect of endomorphin-1 ($P < 0.0001$, 2-factor ANOVA; $n = 5$–7; Fig. 6A).

Adjuvant inflammation and endomorphin-1 responses. One week after intra-articular injection of Freund’s complete adjuvant, the hypoaemic effect of endomorphin-1 in the inflamed knee was significantly attenuated compared with normal con-
trol knee joints \( P < 0.0001 \), 2-factor ANOVA; \( n = 5–7 \); Fig. 6B). This lack of response to the opioid peptide was consistent across the entire dose range tested. Analysis of basal perfusion levels measured at the beginning of each experiment highlighted a noticeable increase in articular vascular resistance in inflamed joints compared with normal control knees (Fig. 7).

**Effects of CTOP on articular basal resistance.** As shown in Fig. 7, topical application of CTOP alone onto rat knees had no observable effect on synovial vascular resistance in the six normal \( (P = 0.37, \text{ 2-tailed paired Student's } t\text{-test}) \) or nine inflamed knee joints \( (P > 0.05) \).

**DISCUSSION**

Morphine has long been known to have antiarthritic properties; however, its long-term use is limited by its potential for multiple side effects and tolerance and addiction issues. Endo-

![Fig. 3](image)

**DIAGRAM**

**Fig. 3.** Immunolocalization of \( \mu \)-opioid receptors in normal (A and B) and 1 wk adjuvant-inflamed (C and D) rat knee synovium. Opioid receptors are clearly distinguishable in normal joints (arrows) but are almost undetectable in inflamed knees. Nerves on which the \( \mu \)-opioid receptors are located appear throughout the tissue matrix and occasionally occur in close apposition to synovial blood vessels (v).

![Fig. 4](image)

**DIAGRAM**

**Fig. 4.** Western blot analysis of normal (A) and adjuvant-inflamed (B) rat synovial tissue. A band consistent with the molecular weight of the \( \mu \)-opioid receptor was detected in samples taken from normal joints. No \( \mu \)-opioid receptor antibody binding was found in inflamed knees.

![Fig. 5](image)

**DIAGRAM**

**Fig. 5.** Time course of effect of endomorphin-1 on normal knee joint vascular resistance with \( 10^{-12} \) mol (■), \( 10^{-11} \) mol (▲), \( 10^{-10} \) mol (◇), and \( 10^{-9} \) mol (●). Maximal hypoaemic effect of the \( \mu \)-opioid receptor agonist tended to occur 6 min after topical application of the drug to the joint. Subsequent time points show the gradual recovery of joint vascular resistance toward control levels. Data are means ± SE.
morphin-1 may be a therapeutically more viable alternative to synthetic opioids for chronic disease treatment as well as in joints 1 wk after induction of adjuvant monoarthritis. Semiquantitative analysis of immunopositive staining in these two groups of animals revealed a significant increase in endomorphin-1 levels in adjuvant-inflamed joints compared with normal control knees. Whether this rise in the amount of endomorphin-1 in arthritic knees is a result of synovial hyper trophy or increased peripheral transport of the neuropeptide is unclear from these results. Nevertheless, this finding is consistent with a radioimmunoassay study in which endomorphin-1 was detected in the synovial extracts of adjuvant-inflamed ankle joints, although the neuropeptide was not found in control joints (13). This lack of measurable endomorphin-1 in control ankles differs from the present investigation, which found low levels of the peptide in normal knee joint synovium. Possible reasons for this discrepancy may be due to differences in antibody affinity or the limit of detection associated with radioimmunoassay. The elevation of endomorphin-1 levels in inflamed joints highlights an intriguing neurogenic response to arthritis progression in which endomorphin-1 accumulates in the synovium to help reduce joint inflammation and pain.

Evidence is emerging to suggest that endomorphin-1 has certain anti-inflammatory properties in joints because peripheral administration of endomorphin-1 to rat knees reduces synovial blood flow and inhibits protein extravasation after an acute inflammatory reaction (2, 25). The present study confirms the hypoaemic response to endomorphin-1 in normal joints and shows that this effect is blocked by coadministration of the \( \mu \)-opioid receptor antagonist CTOP. The reduction in synovial perfusion is probably due to the inhibition of proinflammatory peptides from type IV joint afferents because destruction of these nerves by capsaicin treatment abolishes the hypoaemic effect of endomorphin-1 (2). \( \mu \)-Opioid

Fig. 6. A: comparison of knee joint vascular resistance after topical administration of endomorphin-1 alone (○) and coadministered with CTOP (●). Ability of endomorphin-1 to reduce synovial blood flow is attenuated by the \( \mu \)-opioid receptor antagonist CTOP \( (P < 0.0001 \text{ 2-factor ANOVA;} \; n = 5–7) \). Means ± SE are shown. B: comparison of knee joint vascular resistance after topical administration of endomorphin-1 to normal (○) and adjuvant-inflamed knee joints (●). Hypoaemic effect endomorphin-1 induced in normal joints is completely ameliorated when applied to chronically inflamed knee joints \( (P < 0.0001 \text{ 2-factor ANOVA;} \; n = 5–7) \). Means ± SE are shown.

The present study extends this observation by identifying endomorphin-1 containing nerve fibers in normal knee joints as well as in joints 1 wk after induction of adjuvant monoarthritis. Fig. 7. Comparison of basal vascular resistance of normal (open bars) vs. adjuvant-inflamed (filled bars) knee joints. CTOP had no significant effect on resting vascular resistance in either group of animals. Means ± SE are shown, \( n = 6–9 \). NS, not significantly different.
receptors are known to be present on peripheral afferent nerves (6) where their stimulation causes the deactivation of voltage-gated calcium channels (28). This resulting drop in calcium ion concentration in the afferent nerve terminal inhibits calcium-dependent neurotransmitter release, which includes vasodilator peptides such as substance P (5, 36). This loss of vasodilator dependent neurotransmitter release, which includes vasodilator (6) where their stimulation causes the deactivation of voltage-receptors are known to be present on peripheral afferent nerves.

One week after induction of adjuvant monarthritis, endomorphin-1 had no significant effect on knee joint perfusion. It could be argued that because these joints exhibit enhanced basal vascular resistance (Fig. 7), then the absence of any response to endomorphin-1 could be due to the fact that the synovial blood vessels are maximally vasoconstricted and unable to elicit further reductions in blood flow. However, an earlier study examining adrenergic vasoactivity in this model confirmed that articular blood vessels retain the capacity for ongoing smooth muscle contraction (23). A more likely reason for attenuated endomorphin-1 responsiveness is downregulation of μ-opioid receptors present on type IV joint afferents in the chronically inflamed knee. This was confirmed in the present study where immunohistochemical and Western blot analysis of adjuvant-inflamed synovium failed to find appreciable numbers of μ-opioid receptors. This radical fall in μ-opioid receptor density is probably a consequence of high endomorphin-1 levels in the arthritic joint as revealed by the immunohistochemistry data. Exposure of the joint to abnormally high concentrations of the endogenous peptide likely caused internalization of μ-opioid receptors rendering the joint less sensitive to exogenous administration of additional endomorphin-1. This downregulation of μ-opioid receptors in chronically inflamed tissue differs from what is believed to occur during an acute inflammatory episode. Intraplantar injection of either carrageenan or Freund’s complete adjuvant into the hindpaw of rats causes increased transport of μ-opioid receptors from the dorsal root ganglion to the periphery up to 4 days after treatment, resulting in increased receptor density in the inflamed tissue (12, 14, 38). Interestingly, opioid receptor expression in the dorsal root ganglia of these animals is unchanged (14, 30, 38) so that more receptors are being transported peripherally than are being generated in the cell body. Thus, in the chronic phase of inflammation, the opioid receptor reserve may be exhausted and peripheral receptor number eventually decreases. This process could be an additional factor contributing to the lack of endomorphin-1 activity in the monoarthritic joints described here and it may also explain why chronically inflamed joints are still painful and inflammation continues to progress unabated. Future studies examining the density and expression of μ-opioid receptors in the dorsal root ganglia of arthritic animals are required to validate this prospective outcome.

Experiments were also performed to test the effect of μ-opioid receptor antagonism on basal knee joint blood flow. Here, topical administration of 10−9 mol CTOP had no discernible effect on synovial vascular resistance in either normal control or adjuvant-inflamed joints. This suggests that in normal knees, endomorphin-1 levels are too low to have any tonic influence on articular perfusion and that the role of this peptide in normal vascular control mechanisms is negligible. The lack of response to CTOP in adjuvant monoarthritic joints is probably related to the diminished number of μ-opioid receptors in these knees as demonstrated by the immunohistochemistry and Western blot analysis described here. Thus functional antagonism of μ-opioid receptors is not possible in arthritic knees, because receptor density and possibly functionality is already compromised by complex inflammatory processes.

In summary, the results of this study show that endomorphin-1 acts on μ-opioid receptors to cause a reduction in knee joint blood flow. This hypoaemic effect is absent in week 1 adjuvant-inflamed knees due to downregulation of μ-opioid receptors occurring in response to an accumulation of endomorphin-1 in the inflamed joint. Comparison of these findings with other studies assessing endomorphin-1 in acutely inflamed tissues seems to indicate that the anti-inflammatory efficacy of exogenously administered endomorphin-1 declines as inflammation becomes more chronic. These observations also highlight a potential inadequacy in the endogenous opioid system to ameliorate joint inflammation.

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