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Regulation of Pain Sensitivity in Experimental Osteoarthritis by the Endogenous Peripheral Opioid System

Julia J. Inglis,[1] Kay E. McNamee,[1] Shi-Lu Chia,[1] David Essex,[2] Marc Feldmann,[1] Richard O. Williams,[1] Stephen P. Hunt,[3] and Tonia Vincent[1]

Objective. **OA is the most common joint disease, affecting 10–15% of people over 60 years of age. However, up to 40% of individuals with radiologic damage are asymptomatic. The purpose of this study was to assess the role of the endogenous opioid system in delaying the onset of pain in a murine model of osteoarthritis (OA).**

Methods. **Osteoarthritis was induced by transection of the medial meniscotibial ligament. Pain was assessed by monitoring weight distribution and activity. At various times postsurgery, the opioid receptor antagonists naloxone or peripherally restricted naloxone methiodide were administered, and pain was assessed. Levels of the μ-opioid receptor were assessed in the nerves innervating the joint by real-time reverse transcription–polymerase chain reaction analysis.**

Results. **As in human disease, significant joint damage occurred in mice before the onset of pain. To assess whether delayed pain was partly the result of increased endogenous opioid function, naloxone or naloxone methiodide was administered. Both opioid receptor antagonists led to pain onset 4 weeks earlier than in vehicle-treated mice, indicating a role of the peripheral opioid system in masking OA pain. The expression of the μ-opioid receptor in the peripheral nerves supplying the joint was transiently increased in naloxone-responsive mice.**

Conclusion. **These findings indicate that a temporal induction of μ-opioid receptors in the early stages of OA delays the onset of pain. This is of clinical relevance and may contribute to the assessment of patients presenting with pain late in the disease. Furthermore, it may point to a mechanism by which the body blocks pain perception in moderate states of tissue damage, allowing an increased chance of survival.**

The relationship between pain and tissue damage is complex. In some conditions, such as rheumatoid arthritis, pain is present early in the disease process, presumably due to mediators of inflammation, and this symptom is usually the reason people initially seek medical care (1). In contrast, the pain of pancreatic cancer develops very late in the disease (2). Both early and delayed pain confer advantages to the affected person. Acute pain can be regarded as a mechanism for preventing further damage by limiting one's use of the damaged area in order to foster recovery. Delaying pain, in contrast, increases the chance of survival over the longer term by allowing the injured person to continue functioning for an extended period of time.

Pain often occurs late in the development of osteoarthritis (OA), the most common joint disease, which is characterized by cartilage degeneration (3). The large weight-bearing joints of the lower limbs, the hips, and knees are most typically affected. Patients present with stiffness and activity-related pain, which may be severely debilitating. The generation of pain in the OA joint is poorly understood, and changes in the nociceptive system that are induced by OA have not been well-characterized. Episodic synovitis, which may provoke pain via mediators of inflammation (1), occurs in some patients. However, the correlation between radiologic changes of arthritis and pain is weak, and up to 40% of patients with radiologic evidence of significant joint degeneration are asymptomatic (4). This suggests

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[1]Julia J. Inglis, PhD, Kay E. McNamee, BSc, Shi-Lu Chia, MD, Marc Feldmann, MBBS, PhD, FRS, Richard O. Williams, PhD, Tonia Vincent, MD, PhD: Kennedy Institute of Rheumatology, Imperial College London, London, UK; [2]David Essex, MSc: Imperial College London, London, UK; [3]Stephen P. Hunt, PhD: University College London, London, UK.

Drs. Hunt and Vincent contributed equally to this work.

Address correspondence and reprint requests to Julia J. Inglis, PhD, Kennedy Institute of Rheumatology, Imperial College London, London W6 8LH, UK. E-mail: j.inglis@imperial.ac.uk.

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that OA may be a suitable disease in which to study chronic pain and endogenous mechanisms of pain regulation.

A variety of animal models have been used for studying the pathophysiology of OA (5), although there have been few reported models of experimental OA in rodents. Rodent models that have been used for the study of pain in OA include monosodium iodoacetate, collagenase, or papain injection into the knee joint, or partial meniscectomy (6,7). These models characteristically produce severe and rapidly progressive joint damage, with behavioral changes occurring within days of the procedure. Some models, such as iodoacetate-induced OA, have an early inflammatory component and involve peripheral nerve damage (8).

Recently, a surgical technique that induces a more slowly progressive course of joint degeneration in mice was described (9). In this model, transection of the medial meniscotibial ligament results in instability of the medial meniscus. This leads to a slowly progressive degeneration of the articular cartilage, with little or no synovitis. Meniscal injury is a risk factor for OA in humans, so we used this model to investigate endogenous inhibitory mechanisms in the development of chronic pain associated with the disease. We first established that pain behavior in mice with surgically induced OA is delayed compared with pain behavior associated with joint damage. We then showed that the endogenous peripheral opioid system plays a role in suppressing OA pain.

MATERIALS AND METHODS

Animals. Male mice are more susceptible than female mice to surgically induced OA (10); therefore, adult male C57BL/6 mice ages 10–12 weeks (Harlan, Blackthorn, UK) were used for all studies. Mice were housed in groups of 10 in an environment maintained at an ambient temperature of 21°C (±2°C), with 12-hour cycles of light and dark (7:00 AM to 7:00 PM), and with food and water ad libitum. All experimental procedures were approved by the UK Home Office, and guidelines issued by the International Association for the Study of Pain were followed.

Destabilization of the medial meniscus. Surgery was performed as described previously (9). Mice were anesthetized by intraperitoneal injection of fentanyl/fluanisone (Hypnorm; VetaPharma, Leeds, UK) and midazolam (Hypnovel; Roche, Welwyn Garden City, UK). The ventral portion of the right knee was shaved, and the surgical field was prepared with an antiseptic solution. The procedure was performed using a dissecting microscope. A midline incision was made over the ventral aspect of the knee, and the medial compartment of the knee was entered via a medial parapatellar approach, without displacing the patella or cutting the quadriceps muscle. The medial meniscus was identified, and its anterior horn was released by sharp dissection of the attachments to the tibial plateau using an ophthalmic scalpel. Mobility of the anterior half of the medial meniscus was confirmed by displacement with forceps. Following irrigation with sterile saline solution, the parapatellar window was closed with an absorbable suture (Ethicon, Somerville, NJ), and the skin was closed with fine nonabsorbable sutures (Ethicon). A single dose of buprenorphine analgesic (Vetergesic; Alstoe Animal Health, Sheriff Hutton, UK) was administered postoperatively at 0.1 mg/kg subcutaneously. Sham surgery was performed on the right knee of a separate group of mice and consisted of skin incision and medial capsulotomy only, followed by closure in layers as described above. A third group of mice that did not undergo any surgical procedure served as naive, unoperated controls.

Histologic analysis. Mice were killed after 3 days and 1, 2, 4, 8, and 12 weeks (minimum sample size 5 mice per group). Both knees were removed by sharp division at the proximal femur and distal tibia. The skin and surrounding muscles were then removed without disturbing the joint and its associated ligaments. The knee specimens were fixed in 10% formalin for at least 48 hours and then decalcified in dilute formic acid over a period of 3 weeks. Specimens were then paraffin-embedded, and 4-μm coronal sections were cut with a standard microtome. Sections were cut at 80-μm intervals, yielding 8 samples per joint, and were stained with Safranin O for microscopic inspection and histologic scoring.

Severity of cartilage destruction was assessed histologically using a modification of a previously described 6-point scale (0 = normal, 1 = surface fibrillations, 2 = loss of superficial cartilage, surface delamination, shallow fissures, but no frank ulceration, 3 = ulceration of noncalcified cartilage only, 4 = vertical clefts extending into subchondral bone, 5 = ulceration extending into calcified cartilage but not into subchondral bone, with <80% cartilage loss, and 6 = ulceration extending into subchondral bone and/or >80% cartilage loss) (11). Scoring was performed blindly by 2 observers (JJI and S-LC). The intraobserver and interobserver reliability of this scoring system was assessed by calculating the intraclass coefficient (ICC) as well as the Bland-Altman bias. For the intraobserver reliability, the ICC was 0.888 (alpha level, 2-way random model, absolute agreement, single measure), and the Bland-Altman bias was 0.0227 ± 0.77 (mean ± SEM). For the interobserver reliability, the ICC was 0.947 (alpha level, 2-way random model, absolute agreement, single measure), and the Bland-Altman bias was 0.0961 ± 0.51. These calculated values represent very good observer agreement.

Eight sections from each joint were scored. Each femoral and tibial surface (4 quadrants: the medial femoral condyle and tibial plateau and the lateral femoral condyle and tibial plateau) within each section was scored separately. The maximum score was defined as the single highest score recorded from any joint surface within a single joint in all sections inspected, and this provided an index of peak OA severity. The scores obtained from each surface in any given section were added together to give a section total score, and the summed score was then calculated from the sum of the 3 highest section totals, providing an index of both the severity and the extent of cartilage damage in the joint.

Assessment of weight distribution deficits. Differential distribution of weight was measured using a Linton Incapaci-

tance Tester (MJS Technology, Hertfordshire, UK), as described previously in mice and rats (12,13). Animals were allowed to acclimate to the equipment on at least 2 occasions prior to taking the measurements (n = 8 mice per group). The incapacitance tester is equipped with a small, clear acrylic chamber whose floor is equally divided into 2 electronic weighing scales. The shape of the chamber forces the mice to stand on their hind paws. Mice were maneuvered inside the chamber to stand with 1 hind paw on each scale. The weight that was placed on each hind limb was then measured over a 5-second period. At least 3 separate measurements were made for each animal at each time point, and the result was expressed as the percentage of the weight placed on the operated limb versus the contralateral unoperated limb (operated limb/unoperated limb × 100). Hence, with decreased leaning to the operated side, a decreased percentage of weight distribution was observed.

Cyclooxygenase 2 (COX-2) inhibition and morphine therapy. When all mice displayed ≤70% weight distribution through the operated hind limb (14–16 weeks postsurgery), they were given 30 mg/kg of the COX-2 inhibitor celecoxib (Celebrex; Pfizer, Kent, UK) in 1% hydroxypropyl methylcellulose (HPMC; GlaxoSmithKline, London, UK) by gavage twice daily for 2 days, a dose that has been shown to be effective in inflammatory arthritis (14), or 1% HPMC alone (n = 8 mice per group). Weight distribution was assessed daily, 2 hours after drug administration. Following 2 days of treatment, dosing was then stopped for a further 18 hours (washout period), and measurements of weight distribution were performed again.

An additional group of mice was given 5 or 10 mg/kg of morphine sulfate in saline by intraperitoneal injection (n = 5 mice per group). These doses have previously been shown to be analgesic with minimal sedative effects (15). Weight distribution was assessed before, and 1 hour after, injection.

Assessment of activity/spontaneous behavior. The Laboratory Animal Behavior Observation Registration and Analysis System (LABORAS; Metris, Zoetermeer, The Netherlands) is an automated system that analyzes vibrations evoked by movement of a single rodent in a cage. Pattern-recognition software then classifies and quantifies behaviors, including grooming, activity, climbing, immobility, and feeding (16,17). Our system has 4 cages. Since differences in activity were observed between different batches of mice, we assessed sham-operated and OA mice in parallel (2 sham-operated mice and 2 OA mice per session). Comparisons between individual experiments were not made. Animals were allowed to acclimate to the equipment on 2 occasions prior to taking the measurements. Animals were placed in the LABORAS activity monitor for 3 hours. Mice were studied before, and at various points after, surgery (n = 7 mice per group).

Activating transcription factor 3 (ATF-3) immunohistochemistry. At various points throughout disease development, animals were killed by exposure to CO_2, and the L4–L5 dorsal root ganglia (DRGs) were excised, fixed in 10% formalin, and embedded in paraffin. Nerve damage was assessed using a rabbit anti–ATF-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Antibody detection was performed using an avidin–biotin–peroxidase method (Vector Laboratories, High Wycombe, UK) (18). The number of ATF-3–positive neurons in the DRGs was quantified in 3 sections per mouse by 2 blinded observers (JJI and S-LC).

Naloxone treatment. Naloxone (2.5 mg/kg; Sigma, Poole, UK), naloxone methiodide (2.5 mg/kg; Sigma), or vehicle (phosphate buffered saline) was administered by intraperitoneal injection into naive control mice, which had not undergone any type of surgery, and at various time points after surgery. Weight distribution was assessed before, and 1 hour after, injection, as described above. For activity assessment, mice with surgically induced OA or sham-operated mice were injected with 2.5 mg/kg of naloxone methiodide. Animals were then placed in the LABORAS activity monitor for 3 hours (n = 6 mice per group).

Enzyme-linked immunosorbent assay (ELISA) for β-endorphin levels. At various time points after surgery, mice were killed by CO_2 exposure, and blood was collected by cardiac puncture. Serum was removed following clotting, and β-endorphin levels were assessed by ELISA, according to the manufacturer's instructions (MD Biosciences, Zurich, Switzerland). In addition, sera from mice with collagen-induced arthritis (CIA) were analyzed. To induce CIA, adult male DBA/1 mice were immunized by subcutaneous injection at the base of the tail with 2 × 50 μl of bovine type II collagen (2 mg/ml) in Freund's complete adjuvant (Becton Dickinson, Oxford, UK), as described previously (19). Serum was collected 10 days after the onset of arthritis (n = 5 mice per group).

Real-time reverse transcription–polymerase chain reaction (RT-PCR) for the μ-opioid receptor. The quantification of μ-opioid receptor in the DRGs was determined by real-time RT-PCR. The DRGs innervating the operated knee and the contralateral unoperated knee (L3–L4 lumbar region) were taken from mice at 4, 8, and 16 weeks after surgery to induce OA or after sham surgery. The DRGs were placed in RNAlater (Qiagen, Crawley, UK), and RNA was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. RT for the production of complementary DNA (cDNA) was performed on the RNA samples using an avian myeloblastosis virus RT system from Promega (Southampton, UK), with random hexamer primers, according to the manufacturer's protocol. Amplification of cDNA was performed on a Corbett Life Science Rotor-Gene system (Corbett Life Science, St. Neots, UK), using TaqMan Gene Expression Assays sets from the ABI inventoried library for the μ-opioid receptor (Applied Biosystems, Warrington, UK) and the endogenous control hypoxanthine guanine phosphoribosyltransferase and a 2-step amplification method for the PCR (95°C for 10 minutes followed by 40 cycles of 95°C for 2 seconds and 60°C for 20 seconds). Each run included external standards as positive controls for the standard curve and water without template as negative controls. The cDNA relative concentration in each sample was then calculated automatically by reference to the standard curve using Corbett Rotor-Gene software (version 7.1), with normalization against the endogenous control. Levels were expressed relative to those in sham-operated mice (n = 5 mice per group).

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA). Multiple group means were analyzed by one-way analysis of variance, followed by Dunnett's multiple comparisons test, where appropriate. Unpaired *t*-test was used for experiments involving only 2 groups, and paired *t*-test was used to assess RNA levels within different tissues of the same animal.

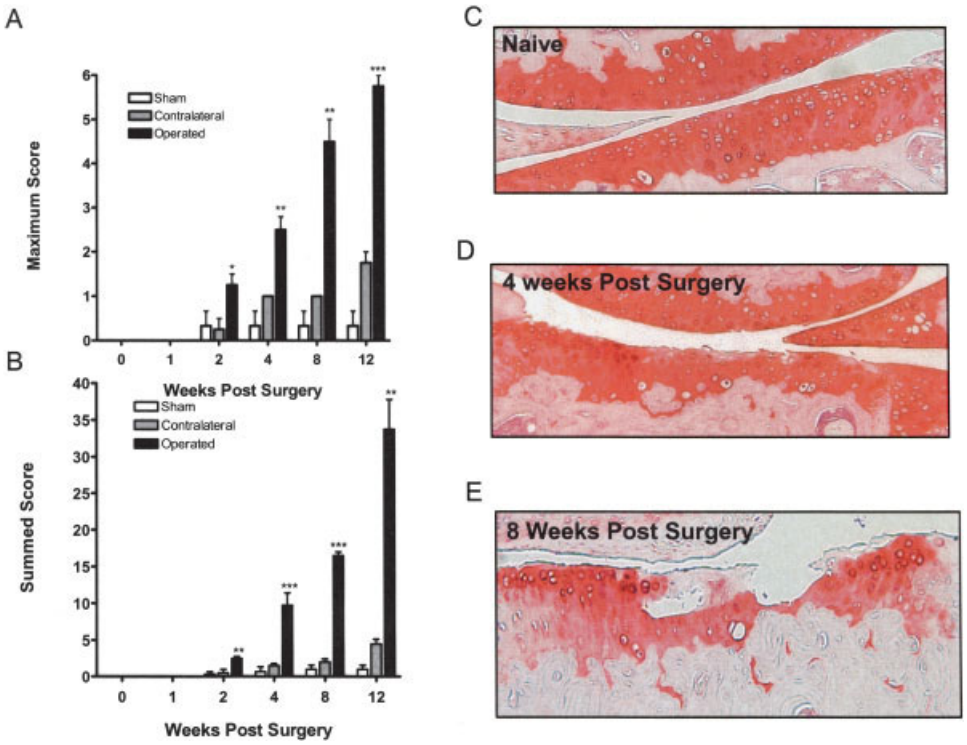


Figure 1. Disease progression in mice with surgically induced osteoarthritis (OA). One group of mice underwent meniscal destabilization of the right knee to induce OA; contralateral knees were unoperated. Another group of mice underwent sham surgery of the right knee. **A,** Maximum histologic scores in all sections of each knee joint obtained at the indicated time points (see Materials and Methods for details). **B,** Summed scores for each knee (obtained by adding the score from each quadrant of the 3 most severely affected sections). Values are the mean and SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$ versus sham-operated controls. **C–E,** Representative histology sections obtained from surgically naive mice (**C**) and from mice subjected to meniscal destabilization assessed at 4 weeks (**D**) and 8 weeks (**E**) following surgery (original magnification × 250).

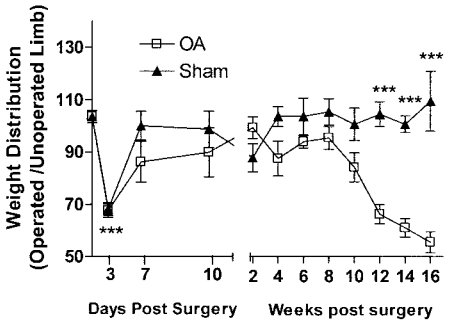
RESULTS

Histologic progression of cartilage degeneration in murine OA. Initial studies were performed to determine whether surgically induced OA was a useful model for the study of endogenous inhibitory mechanisms of pain. In order to do this, we investigated the relationship between joint damage and pain in surgically induced OA. At various time points after surgery, mice were killed, and the operated and contralateral unoperated knees were collected. Histologic scoring of the knees was performed, and the maximum scores (Figure 1A) and the sum of the 3 most damaged sections (summed score) (Figure 1B) were calculated.

There was no evidence of cartilage damage in naive, unoperated control mice (no surgery) (Figure 1C) or at 3 days or 1 week postsurgery in the mice subjected to surgical induction of OA. At 2 weeks postsurgery, a small, yet statistically significant, increase in histology scores was noted in the operated knee as compared with the unoperated and sham-operated knees. The damage in the operated knee increased further at 4 weeks (Figure 1C), 8 weeks (Figure 1D), and 12 weeks postsurgery, reflecting progressive joint disease. There was no difference in serial weight gain between the operated, sham-operated, and naive groups of mice (data not shown), indicating that the degeneration observed was not a result of a greater load distribution through increased body weight and that mice were not adversely affected by the surgery.

Delayed onset of pain in murine OA. Next, we assessed temporal changes in pain behavior after surgically induced OA. Pain associated with unilateral arthritis can be measured by changes in weight distribution between the operated and contralateral, unoperated, hind limbs. This was measured using a Linton Incapacitance Tester. The apparatus chamber is designed to

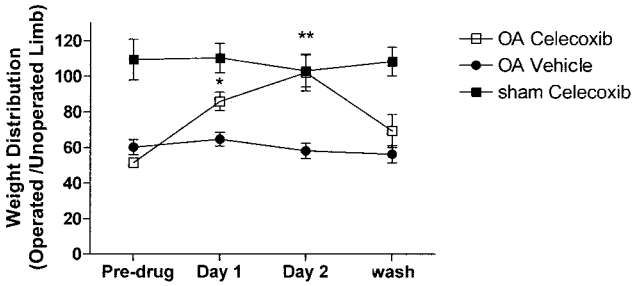
A



B

	Naive		3 Days		4 Weeks		8 Weeks		16 Weeks	
Limb	L	R	L	R	L	R	L	R	L	R
OA Mean +/- SD	10.7 +/- 1.3	10.0 +/- 1.5	13.8 +/- 0.8	7.3 +/- 0.8***	10.1 +/- 1.9	8.7 +/- 1.1	11.71 +/- 2.3	9.8 +/- 1.7	17.3 +/- 2.9	9.6 +/- 2.4***
SHAM Mean +/- SD	9.9 +/- 2.3	10.2 +/- 1.1	14.4 +/- 1.4	9.0 +/- 2.6***	9.4 +/- 1.4	9.7 +/- 1.4	10.7 +/- 1.2	11.2 +/- 1.6	12.5 +/- 0.9	12.9 +/- 1.9

C



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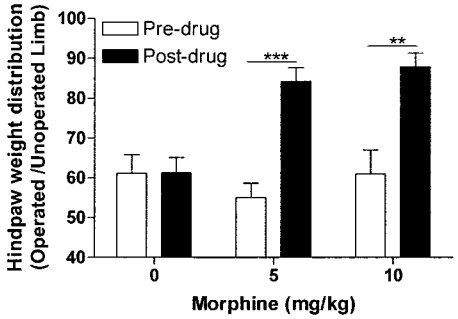

Figure 2. Late onset of pain in mice with surgically induced osteoarthritis (OA). Weight distribution as a measure of pain was assessed in mice before and after surgical induction of OA or sham operation. **A,** Weight distribution in mice with surgically induced OA and sham-operated mice, expressed as the percentage of the weight placed on the operated limb versus the contralateral unoperated limb. **B,** Weight distribution raw data (grams) for the right and left sides, as determined at the indicated time points before (naive) and after surgery. **C,** Weight distribution at 14 weeks postsurgery (when all mice displayed $\leq$70% weight distribution through the operated hind limb), as assessed before treatment, on day 1 and day 2 of treatment with celecoxib or vehicle, and following an 18-hour washout period. **D,** Weight distribution at 14 weeks postsurgery, as assessed before treatment and 1 hour after treatment with 5 mg/kg or 10 mg/kg of morphine sulfate or vehicle. Values in **A**, **C**, and **D** are the mean $\pm$ SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$ versus sham-operated controls.

force the mouse onto its hind limbs, with each limb on a separate weighing scale. The weight transmitted through the operated hind limb is then assessed and expressed as a percentage of the weight transmitted through the contralateral, unoperated, limb (Figure 2A). Raw data for the weight transmitted through the operated (right) and unoperated (left) limb are shown in Figure 2B.

Assessment of weight distribution showed a significant shift in weight away from the operated limb at 3 days postsurgery (Figures 2A and B). This was probably due to the early postoperative injury response. However, both groups of mice returned to normal weight distribution by 1 week postsurgery. Thereafter, sham-operated mice maintained equal weight distribution throughout the period of study (16 weeks). In contrast, a statistically significant reduction in weight distribution occurred in OA mice from 12 weeks of age, compared with mice with sham surgery (Figures 2A and B). By 14 weeks postsurgery, all mice displayed unequal weight distribution. From these data, we deduced that pain behavior was delayed by 10 weeks relative to the histologic evidence of tissue damage.

Reversal of changes in weight distribution by analgesic therapy. To confirm that the changes in weight distribution observed following surgery were a true indicator of pain associated with the disease process, we assessed the ability of the analgesic COX-2 inhibitor celecoxib to reverse the deficit in mice at 14 weeks postsurgery (Figure 2C). Celecoxib therapy started to reduce the weight distribution changes after 1 day of therapy (2 doses) and completely reversed the weight distribution defects after 2 days of therapy (4 doses). At

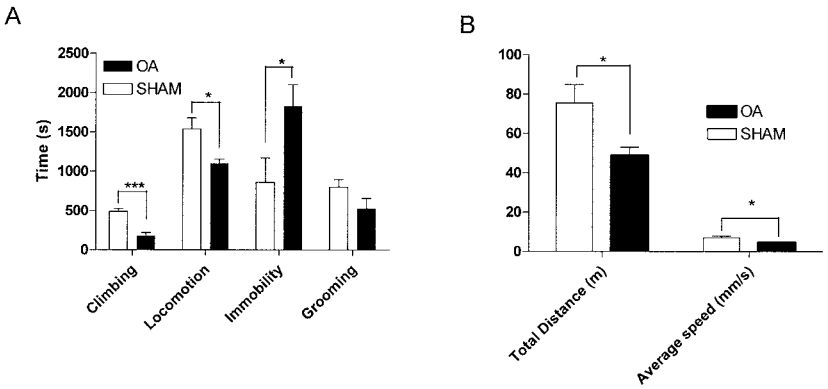

Figure 3. Spontaneous changes in the behavior of mice with surgically induced osteoarthritis (OA). At 8 weeks after surgical induction of OA or sham operation, spontaneous activity was assessed over a 3-hour period using the Laboratory Animal Behavior Observation Registration and Analysis System activity monitor. **A,** Time spent climbing, in locomotion, immobile, and grooming (in seconds). **B,** Total distance traveled (in meters) and average speed (in mm/second). Values are the mean and SEM. $* = P < 0.05$; $*** = P < 0.001$.

18 hours after cessation of celecoxib therapy, the weight distribution deficits returned to pretreatment levels. Likewise, a single dose of 5 mg/kg or 10 mg/kg of morphine sulfate reversed the weight distribution deficits at 1 hour posttreatment (Figure 2D). These data indicate that weight distribution in this model is a good indicator of pain derived from the diseased joint and can be reversed by treatment with well-established analgesics.

Changes in activity and spontaneous behavior following induction of OA. To measure spontaneous activity, we used the automated activity monitor LABORAS. This system assesses spontaneous behaviors, such as grooming, climbing, and feeding, of mice housed singly by assessing the vibrations the mouse transmits through the floor of the cage (16). We have previously shown that this system is useful in the study of pain from inflammatory arthritis and that there was modification of behavior upon treatment with analgesics (14,20). Spontaneous behavior patterns were comparable in the operated and sham-operated mice at 2 weeks and 4 weeks postsurgery (data not shown).

However, at 8 weeks postsurgery, significant differences in spontaneous activity between the 2 groups were observed (Figure 3). Mice with OA spent significantly less time climbing and moving and spent more time immobile (Figure 3A). This resulted in significantly less distance traveled and a decreased average speed of movement in the operated mice versus the sham-operated mice (Figure 3B). Grooming behavior was unaffected by surgery, indicating general well-being in the mice. These data indicate a disparity between pain behavior and joint damage by 6 weeks postsurgery, which is 4 weeks earlier than the changes in weight bearing.

Role of the endogenous opioid system in OA pain. Having established a disparity between joint damage and pain onset in this model, we investigated changes in the endogenous opioid system as a possible mediator of the delayed pain behaviors. We administered the opioid antagonist naloxone (penetrates the peripheral and central nervous systems) or the peripherally restricted (non–CNS-penetrant) antagonist naloxone methiodide to mice at various time points following surgically induced OA and then assessed weight distribution using the Linton Incapacitance Tester. As noted above, changes in weight distribution in the operated limb were expressed as a percentage of that in the unoperated limb (Figure 4A).

As seen previously, statistically significant changes in weight distribution were observed in vehicle-treated OA mice from 12 weeks, but not 8 weeks, postsurgery (Figure 4A). Naloxone administration 1 hour prior to assessment induced weight distribution changes in mice at 8 weeks postsurgery (Figure 4A). A similar response was observed with administration of the non–CNS-penetrant naloxone methiodide, with significantly altered weight distribution observed from 8 weeks postsurgery (Figure 4A). This indicated that the action of endogenous opiates on inhibition of pain behavior in this model was restricted to the peripheral nervous system; hence, the peripherally restricted naloxone me-

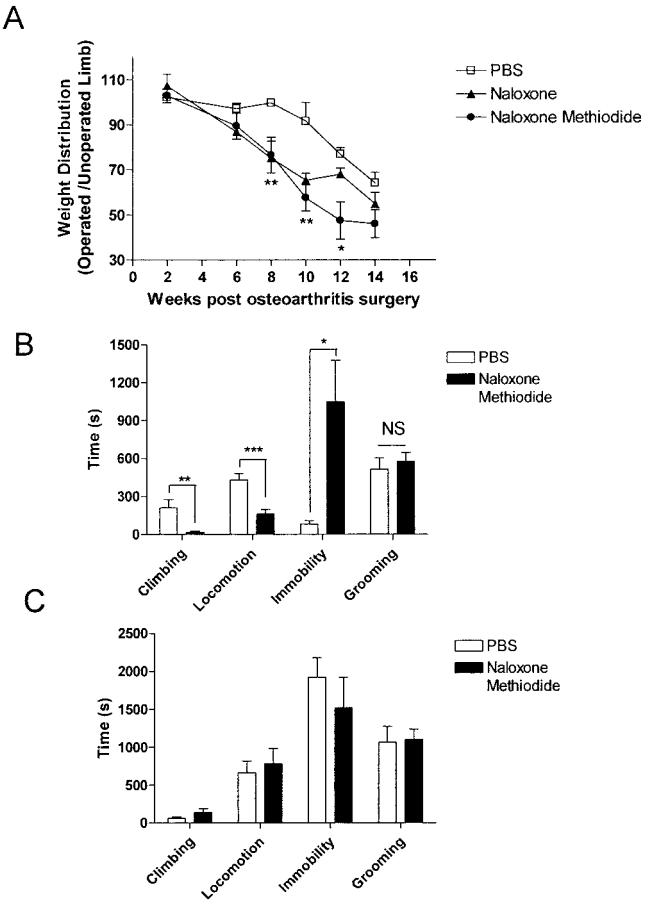

Figure 4. Inhibition of the endogenous opioid system and unmasking of pain in mice with surgically induced osteoarthritis (OA). Mice were treated with 2.5 mg/kg of naloxone, naloxone methiodide, or phosphate buffered saline (PBS) vehicle at various time points after surgery, and weight distribution was assessed before, and 1 hour after, treatment (**A**). At 8 weeks after surgically induced OA (**B**), or sham surgery (**C**), mice were treated with 2.5 mg/kg of naloxone methiodide or PBS vehicle and placed in the Laboratory Animal Behavior Observation Registration and Analysis System activity monitor for 3 hours. Time spent climbing, in locomotion, immobile, and grooming (seconds) was quantified. Values are the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$ versus PBS-treated controls. NS = not significant.

thiodide was used for all further experiments. Naloxone administration did not alter weight distribution in the sham-operated mice (data not shown). Taken together, these data indicate that increased activity of the endogenous opioid system suppresses pain behavior during the progression of OA.

We subsequently assessed the influence of the peripheral opioid system on spontaneous behavior using the LABORAS activity monitor, as described above. Naloxone methiodide or vehicle was administered to the operated and sham-operated mice at 8 weeks postsurgery. The mice showed increased pain behavior upon naloxone injection when tested 3 days prior to this study. Mice were monitored for 3 hours using the LABORAS activity monitor. Naloxone methiodide administration to mice with OA resulted in significantly decreased time spent climbing and in locomotion and increased immobility as compared with pretreatment activity (Figure 4B), while sham-operated mice showed no behavioral changes with naloxone methiodide treatment (Figure 4C). This suggests that there is an increased peripheral opioid drive in mice with OA that is absent in uninjured mice.

Mechanism of the enhanced opioid action in OA. To investigate the mechanism of the increased peripheral opioid drive in mice with OA, we assessed whether systemic induction of an endogenous μ-opioid receptor agonist, β-endorphin, could be detected in mice with OA (Figure 5A). No increase in β-endorphin levels was observed at any time assessed postsurgery. This was in contrast to the findings in mice with CIA, in which significant levels of β-endorphin were detected in the serum. These data indicate that if systemic induction of β-endorphin occurs in surgically induced OA, it is at a very low level.

Next, we examined the levels of messenger RNA (mRNA) for the μ-opioid receptor in the sensory neurons that innervate the joint (Figure 5B). Real-time RT-PCR was performed on RNA extracted from the L3–L4 dorsal root ganglia innervating the operated and contralateral limbs at 4 weeks (when no pain was detected), 8 weeks (pain-free mice that developed pain when given naloxone methiodide), and 16 weeks (when all mice displayed pain) postsurgery. Levels were expressed as the fold change relative to sham-operated mice at each time point (Figure 5B). A 3-fold increase in μ-opioid receptor expression was observed in the ipsilateral DRGs at 8 weeks postsurgery in mice responsive to naloxone methiodide as compared with the sham-operated mice. No changes were detected at 4 weeks (before development of pain) or 16 weeks (after pain behavior had been established) after surgery.

To rule out the possibility that neuronal death accounted for the reduction in μ-opioid receptor expression, we assessed ATF-3 expression, a marker of neuronal damage, in the sensory neurons that innervate the joint. No ATF-3 expression was detected by immunohistochemistry of the L3–L4 DRGs up to 16 weeks postsurgery (data not shown), indicating the non-neuropathic nature of this OA model.

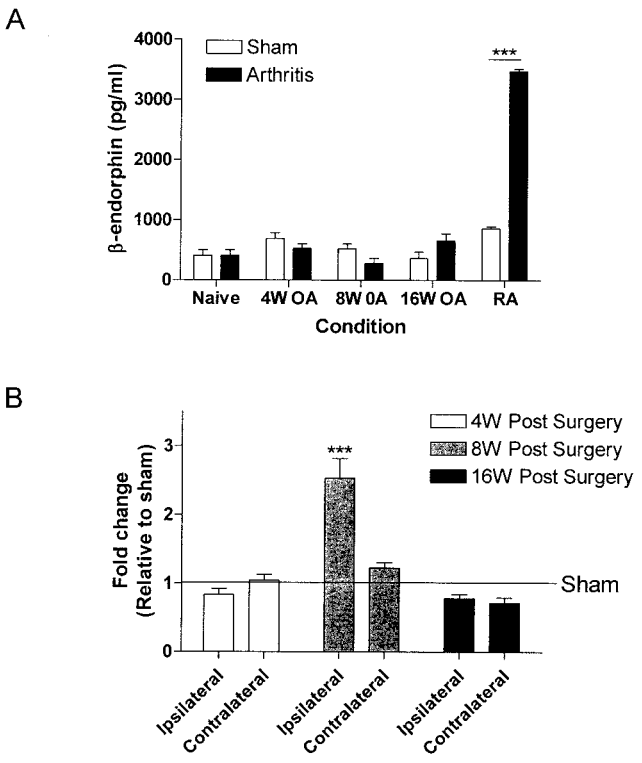

Figure 5. Increased opioid receptor expression in naloxone-responsive mice following surgically induced osteoarthritis (OA). Serum levels of β-endorphin were assessed at 4, 8, or 16 weeks after surgically induced OA or sham surgery and were compared with those from mice with a collagen-induced arthritis (**A**). Levels of mRNA for the μ-opioid receptor in the dorsal root ganglia that innervate the operated knee were assessed at 4, 8, and 16 weeks after surgically induced OA (**B**). Levels of mRNA for μ-opioid receptors were expressed as the fold change relative to sham-operated mice (horizontal line). Values are the mean and SEM. *** = $P < 0.001$ versus sham-operated controls.

DISCUSSION

In these studies, we have demonstrated that in a mouse model of OA, pain behaviors develop several weeks after detectable histologic damage to the joint and that this delay is partly due to inhibition by peripherally active endogenous opiates acting on sensory fibers that innervate the injured knee joint. The lag between the appearance of pain behavior and cartilage damage in this model is in contrast to that in models of inflammatory arthritis and chemically induced OA, in which damage (inflammatory) and increased pain occur simultaneously (5). Indeed, in our study, a significant proportion of mice with histologic evidence of severe cartilage damage displayed no pain behavior, a phenomenon that is common clinically (4). We confirmed that the observed changes in weight distribution reflected pain, since the analgesics celecoxib and morphine sulfate reversed the behavioral and weight-bearing deficits. COX-2 inhibitors are standard analgesics for the treatment of OA pain (21), and their efficacy demonstrated here indicates that this model of surgically induced OA is useful for the assessment of analgesic therapy. Following withdrawal of therapy, the deficits in weight distribution returned to pretreatment values within 18 hours, indicating that COX-2 inhibition provides only short-term analgesia.

Spontaneous activity was reduced in OA mice from 8 weeks postsurgery, which is 4 weeks prior to the onset of pain, as assessed by alterations in weight distribution. No change in grooming behavior was observed. This is in contrast to the findings in mice with CIA, a model of inflammatory arthritis, in which a significant decrease in grooming was observed (14). A lack of grooming is thought to represent sickness behavior due to systemic cytokine release (22). This suggests that with the OA model, the observed differences in activity/behavior are due to local, joint-specific disability rather than systemic illness. The temporal differences between the detection of pain onset according to physical activity and weight distribution may be due to the sensitivity of each measuring system used. It appears that spontaneous changes in behavior occur when there is a low level of pain, but higher levels of pain are required for the detection of changes in weight distribution using the incapacitance tester. This most likely reflects the fact that weight distribution measurements made with the incapacitance tester were obtained when the animal was bearing weight but was inactive, whereas measurements made with the LABORAS activity monitor were obtained when the animal was in motion.

Pain behavior could be unmasked in mice 8 weeks after the establishment of OA and 4 weeks before pain behavior generally becomes detectable with the weight-bearing test. In this regard, we observed an increase in μ-opioid receptor expression in mice at 8 weeks postsurgery. Interestingly, no change in receptor levels was observed between mice at 4 weeks postsurgery, before the development of pain and when naloxone methiodide had no effect, as compared with 16 weeks postsurgery, when pain was already evident, indicating a transient increase in receptor expression. This finding is consistent with the findings of our behavioral studies, which showed that the opioid antagonist had no effect at 4 weeks and 16 weeks postsurgery, but unmasked pain at 8 weeks postsurgery. Opioid receptor mRNA induction was only observed in the DRGs inner-

vating the injured knee, implying that a mediator produced in the joint may induce receptor expression. In models of inflammation, induction of the peripheral opioid receptor is observed in the DRGs through an action in the joint, since blocking sciatic nerve conduction prevents the arthritis-induced receptor upregulation (23). The increase in μ-opioid receptor expression in inflammatory arthritis is not sufficient to block pain, since hyperalgesia occurs at the onset of inflammation (23). However, since there is little overt inflammation in OA, it is probable that the observed increase in receptor expression is sufficient to allow the endogenous opioid system to inhibit the pain behavior.

The late onset of pain observed in mice with surgically induced OA is similar to that observed in a mouse model of pancreatic cancer (24), in which the central opioid system was shown to inhibit pain at earlier stages of the disease. However, unlike the pain of pancreatic cancer, the pain of OA could be unmasked by both CNS-penetrant and non–CNS-penetrant opioid antagonists. This indicates that inhibition of OA-associated pain is mediated by the peripheral, rather than the central, opioid system. This highlights the diversity of the endogenous opioid system in suppressing pain in disease states. There are many differences between the 2 models that may contribute to the induction of the different inhibitory pathways. In pancreatic cancer, the injured organ is static, whereas in OA, the joint is mobile and load bearing. Interestingly, reduced activity of mice with surgically induced OA was observed from 8 weeks postsurgery, 4 weeks prior to the occurrence of pain on weight bearing. It is possible that reduced activity is a trigger for the induction of μ-opioid receptors at 8 weeks postsurgery, resulting in a delayed onset of pain on weight bearing.

It has been shown that proinflammatory cytokines and nerve growth factor can induce μ-opioid receptor expression in inflammatory conditions (23,25,26) and may be involved in the transient increase in opiate receptors in OA. One could envisage that proinflammatory cytokines produced locally in the joint act on the nerves to induce receptor expression and a transient analgesia. The decrease in μ-opioid receptors to basal levels at the latest time points (16 weeks) may reflect the development of nerve damage in the OA joint (27). However, we assessed nerve damage in mice with surgically induced OA by monitoring ATF-3 expression in DRG neuron cell bodies and saw no damage at any stage of the model, indicating that this is unlikely to be the cause of the normalization of μ-opioid receptor expression later in OA.

Opiates could potentially be released from the hypothalamus or produced locally by cells of the joint. However, we did not detect any increase in serum β-endorphin in the OA model, which casts doubt on a systemic induction. This is in contrast to CIA, in which systemic induction of β-endorphin was observed. Again, this highlights the fact that in inflammatory arthritis, the inflammation becomes systemic, whereas in OA, the disease is confined to the joints. There is some evidence supporting a role of a local opioid-inhibitory system in the joint. Nociceptive fibers innervating the joint are known to express opioid receptors (28) and β-endorphin and met-endorphin are found in the synovial tissues of patients with OA (29). Intraarticular opiates are highly effective in treating OA pain, indicating that there are opioid-responsive fibers within the joint (30–33). Moreover, naloxone administration into the knee joint exacerbates pain following knee surgery, indicating a tonic inhibition by the endogenous peripheral opioid system (32).

In summary, we have shown that surgically induced OA is a powerful model for the study of endogenous analgesic mechanisms. Our findings indicate that OA pain and the behavioral response to pain are regulated in part by μ-opioid receptors in the peripheral nervous system. This is of significant clinical relevance, since most OA patients present with pain late in the disease, when significant damage has already occurred. In evolutionary terms, local inhibition of pain would allow the animal to continue normal life despite mild tissue damage and would impart an advantage for survival.

AUTHOR CONTRIBUTIONS

Dr. Inglis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Inglis, Feldmann, Hunt, Vincent.

Acquisition of data. Inglis, McNamee, Chia, Essex.

Analysis and interpretation of data. Inglis, McNamee, Feldmann, Vincent.

Manuscript preparation. Inglis, Feldmann, Williams, Hunt, Vincent.

Statistical analysis. Inglis, McNamee, Chia.

ROLE OF THE STUDY SPONSOR

GlaxoSmithKline had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

REFERENCES

1. Zatarain E, Strand V. Monitoring disease activity of rheumatoid arthritis in clinical practice: contributions from clinical trials [review]. Nat Clin Pract Rheumatol 2006;211:611–8.

2. Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain [review]. Nat Rev Cancer 2002;23: 201–9.
3. Wieland HA, Michaelis M, Kirschbaum BJ, Rudolphi KA. Osteoarthritis: an untreatable disease? [review] [published erratum appears in Nat Rev Drug Discov 2005;4:543]. Nat Rev Drug Discov 2005;4:331–44.
4. Kidd BL. Osteoarthritis and joint pain [review]. Pain 2006;123:6–9.
5. Ameye LG, Young MF. Animal models of osteoarthritis: lessons learned while seeking the "Holy Grail" [review]. Curr Opin Rheumatol 2006;18:537–47.
6. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W. Pralnacasan, an inhibitor of interleukin-1β converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis Cartilage 2003;11:738–46.
7. Pritzker KP. Animal models for osteoarthritis: processes, problems and prospects [review]. Ann Rheum Dis 1994;53:406–20.
8. Ivanavicius SP, Ball AD, Heapy CG, Westwood FR, Murray F, Read SJ. Structural pathology in a rodent model of osteoarthritis is associated with neuropathic pain: increased expression of ATF-3 and pharmacological characterisation. Pain 2007;128:272–82.
9. Glasson SS, Askew R, Sheppard B, Carito BA, Blanchet T, Ma HL, et al. Characterization of and osteoarthritis susceptibility in ADAMTS-4–knockout mice. Arthritis Rheum 2004;50:2547–58.
10. Ma HL, Blanchet TJ, Peluso D, Hopkins B, Morris EA, Glasson SS. Osteoarthritis severity is sex dependent in a surgical mouse model. Osteoarthritis Cartilage 2007;15:695–700.
11. Chambers MG, Bayliss MT, Mason RM. Chondrocyte cytokine and growth factor expression in murine osteoarthritis. Osteoarthritis Cartilage 1997;5:301–8.
12. Barton NJ, McQueen DS, Thomson D, Gauldie SD, Wilson AW, Salter DM, et al. Attenuation of experimental arthritis in TRPV1R knockout mice. Exp Mol Pathol 2006;81:166–70.
13. Fernihough J, Gentry C, Malcangio M, Fox A, Rediske J, Pellas T, et al. Pain related behaviour in two models of osteoarthritis in the rat knee. Pain 2004;112:83–93.
14. Inglis JJ, Notley CA, Essex D, Wilson AW, Feldmann M, Anand P, et al. Collagen-induced arthritis as a model of hyperalgesia: functional and cellular analysis of the analgesic actions of tumor necrosis factor blockade. Arthritis Rheum 2007;56:4015–23.
15. Pomonis JD, Boulet JM, Gottshall SL, Phillips S, Sellers R, Bunton T, et al. Development and pharmacological characterization of a rat model of osteoarthritis pain. Pain 2005;114:339–46.
16. Quinn LP, Stean TO, Chapman H, Brown M, Vidgeon-Hart M, Upton N, et al. Further validation of LABORAS using various dopaminergic manipulations in mice including MPTP-induced nigro-striatal degeneration. J Neurosci Methods 2006;156:218–27.
17. Van de Weerd HA, Bulthuis RJ, Bergman AF, Schlingmann F, Tolboom J, Van Loo PL, et al. Validation of a new system for the automatic registration of behaviour in mice and rats. Behav Processes 2001;53:11–20.
18. Shu SY, Ju G, Fan LZ. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 1988;85:169–71.
19. Williams RO. Models of rheumatoid arthritis. Ernst Schering Res Found Workshop 2005;50:89–117.
20. Williams RO, Inglis JJ, Simelyte E, Criado G, Sumariwalla PF. Analysing the effect of novel therapies on cytokine expression in experimental arthritis [review]. Int J Exp Pathol 2005;86:267–78.
21. Clemett D, Goa KL. Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain [published erratum appears in Drugs 2001;61:498]. Drugs 2000;59:957–80.
22. Cirulli F, De Acetis L, Alleva E. Behavioral effects of peripheral interleukin-1 administration in adult CD-1 mice: specific inhibition of the offensive components of intermale agonistic behavior. Brain Res 1998;791:308–12.
23. Puehler W, Zollner C, Brack A, Shaqura MA, Krause H, Schafer M, et al. Rapid upregulation of μ opioid receptor mRNA in dorsal root ganglia in response to peripheral inflammation depends on neuronal conduction. Neuroscience 2004;129:473–9.
24. Sevcik MA, Jonas BM, Lindsay TH, Halvorson KG, Ghilardi JR, Kuskowski MA, et al. Endogenous opioids inhibit early-stage pancreatic pain in a mouse model of pancreatic cancer. Gastroenterology 2006;131:900–10.
25. Kraus J, Borner C, Giannini E, Hollt V. The role of nuclear factor κB in tumor necrosis factor-regulated transcription of the human μ-opioid receptor gene. Mol Pharmacol 2003;64:876–84.
26. Vidal EL, Patel NA, Wu G, Fiala M, Chang SL. Interleukin-1 induces the expression of μ opioid receptors in endothelial cells. Immunopharmacology 1998;38:261–6.
27. Kohno T, Ji RR, Ito N, Allchorne AJ, Befort K, Karchewski LA, et al. Peripheral axonal injury results in reduced mu opioid receptor pre- and post-synaptic action in the spinal cord. Pain 2005;117:77–87.
28. Bergstrom J, Ahmed M, Li J, Ahmad T, Kreicbergs A, Spetea M. Opioid peptides and receptors in joint tissues: study in the rat. J Orthop Res 2006;24:1193–9.
29. Bender T, Barna I, Geher P. Synovial immunoreactive β-endorphin levels in rheumatoid arthritis and osteoarthritis [letter]. Clin Exp Rheumatol 1999;17:630.
30. Likar R, Schafer M, Paulak F, Sittl R, Pipam W, Schalk H, et al. Intraarticular morphine analgesia in chronic pain patients with osteoarthritis. Anesth Analg 1997;84:1313–7.
31. Le Loet X, Pavelka K, Richarz U. Transdermal fentanyl for the treatment of pain caused by osteoarthritis of the knee or hip: an open, multicentre study. BMC Musculoskelet Disord 2005;6:31.
32. Stein C. Peripheral mechanisms of opioid analgesia [review]. Anesth Analg 1993;76:182–91.
33. Stein A, Yassouridis A, Szopko C, Helmke K, Stein C. Intraarticular morphine versus dexamethasone in chronic arthritis. Pain 1999;83:525–32.