Up-regulation of δ-opioid receptors and κ-opioid receptors in the skin of fibromyalgia patients

ARTICLE *in* ARTHRITIS & RHEUMATOLOGY · JULY 2007

Impact Factor: 7.76 · DOI: 10.1002/art.22735 · Source: PubMed

7 AUTHORS, INCLUDING:

Uwe Wollina
Hospital Dresden-Friedrichstadt
1,697 PUBLICATIONS **6,937** CITATIONS
SEE PROFILE

Renate E Gay
University of Zurich
300 PUBLICATIONS **13,026** CITATIONS
SEE PROFILE

Steffen Gay
University of Zurich
791 PUBLICATIONS **30,401** CITATIONS
SEE PROFILE

Haiko Sprott
University of Zurich
131 PUBLICATIONS **1,746** CITATIONS
SEE PROFILE

ARTHRITIS & RHEUMATISM
Vol. 56, No. 7, July 2007, pp 2464–2466

CONCISE COMMUNICATION

DOI 10.1002/art.22735

Up-regulation of δ-opioid receptors and κ-opioid receptors in the skin of fibromyalgia patients

Fibromyalgia (FM) is a chronic musculoskeletal pain disorder of unknown etiology seen predominantly in women. Even though the origin of FM is still unclear, certain pain-modulating systems, such as the neuropeptide system or the opioid system, may play a role in the pain-controlling mechanisms. Opioids exert their effects through interaction with the opioid receptors (ORs), μ-OR (MOR), κ-OR (KOR) (1,2), and δ-OR (DOR) (3). An interaction between endogenous opioids and their receptors is essential for opioid-mediated analgesic effects. There are different types of endogenous opioid peptides: endorphins, enkephalins, and dynorphins (4,5). At present, most opioids used in clinical practice are agonists of MOR (6). However, the risk of unpleasant or life-threatening effects, and of tolerance and physical dependence, typically restrict opioid therapy for pain management. In contrast, it has been suggested that selective agonists that activate DOR produce antinociception with minimal side effects (7). Previous studies have shown that opioid antinociception might be initiated by activation of ORs outside the central nervous system (CNS) (8–10), indicating that targeting of peripheral ORs could be useful in the treatment of chronic pain.

We hypothesized that the expression of ORs in the skin of FM patients may be abnormal compared with healthy controls, due to persistent pain and inadequate treatment. Considering that the expression of ORs in skin might be correlated with pain in FM, we investigated the expression of messenger RNA (mRNA) for ORs in the skin of FM patients compared with normal controls, by real-time quantitative polymerase chain reaction (PCR). Moreover, levels of pain and physical function in each FM patient were evaluated using the Fibromyalgia Impact Questionnaire (FIQ) (11). The subset of FIQ items that measure physical function are rated on a scale of 0–3, where 0 = able to do and 3 = never able to do.

Skin biopsy specimens were obtained from 25 women with FM who had a mean ± SEM age of 46.6 ± 2.5 years (range 34–61 years) and a mean ± SEM disease duration of 18.7 years (range 2–36 years). Ten women (with a mean ± SEM age of 45.0 ± 4.4 years) who did not have muscular aches and pains and considered themselves to be healthy were used as controls. The control samples were the same as were used in a previously reported study (10). Patients were seen at University Hospital and at the Department of Dermatology and Allergy of the University of Jena (Jena, Germany) and were diagnosed as having FM according to the American College of Rheumatology criteria (12). Normal brain tissue (obtained from the occipital cortex area of cadavers <4 hours after death) provided by the Institute of Neuropathology was used as the positive control for reverse transcriptase (RT)–PCR. Skin tissue samples were surgically removed from the left deltoid area under local anesthesia. All procedures were approved by the local ethics committees.

Total RNA (TRIzol LS reagent; Gibco BRL, Basel, Switzerland) isolated from tissue samples was reverse-transcribed (300 ng of total RNA per 10 μl of RT reaction) using random hexanucleotide primers and RT reaction mixture (TaqMan transcription kit; PE Applied Biosystems, Rotkreuz, Switzerland). PCRs were performed with a TaqMan cycler (PE Applied Biosystems) for 10 minutes at 25°C, 30 minutes at 48°C, and 5 minutes at 95°C. Primers and probes were chosen with the assistance of the Primer Express program (PE Applied Biosystems) (10). The PCR mixture (50-μl total volume) consisted of forward and reverse primers (900 n*M*), TaqMan probe (200 n*M*), and TaqMan universal Master Mix (25 μl of 2× reaction mixture). The PCR universal Master Mix and predeveloped 18S ribosomal RNA were also obtained from PE Applied Biosystems. Gene sequences were amplified in a cycler (TaqMan; PerkinElmer, Rotkreuz, Switzerland) using gene-specific oligonucleotide primers (10). Negative controls included no template control and RNA control to check for genomic contamination. Complementary DNA (cDNA) from normal brain tissue samples was used as the positive control. Relative expression levels of mRNA for ORs were calculated using the comparative threshold method, as recommended by the manufacturer (PE Applied Biosystems), after confirming that ORs and 18S cDNA were amplified with the same efficiency.

Statistical analysis was performed using SPSS (SPSS, Chicago, IL). Real-time PCR results were analyzed by nonparametric Mann-Whitney test. The mean ± SD for each variable was calculated. *P* values less than 0.05 were considered significant.

The FIQ includes physical, psychological, social, and global well-being domains. Our results showed that health-related quality of life was significantly decreased in FM patients. Most of the patients had increased pain, fatigue, and morning tiredness, as well as a decrease in physical function. The mean ± SD scores for each of the FIQ items in a subset of FM patients are shown in Table 1. None of the FIQ items were correlated with age or time since diagnosis.

DOR and KOR genes were detectable at between 27 and 35 cycles in all skin tissue samples examined, although levels of expression varied among patients. Figure 1 shows expression levels of KOR and DOR in the skin of FM patients compared with healthy controls. In FM patients the mean ± SD expression level of DOR in the skin was 59.0 ± 5.9–fold higher than that in healthy controls ($P = 0.001$), and the mean ± SD expression level of KOR was 21.1 ± 6.1–fold higher than that in healthy controls ($P = 0.009$). MOR was

Table 1. Mean ± SD scores on individual items in the Fibromyalgia Impact Questionnaire (FIQ) in the fibromyalgia patients*

Physical function	15.1 ± 3.4
Feel good	10.0 ± 5.2
Work missed	3.3 ± 2.1
Job ability	66.0 ± 4.0
Pain	78.0 ± 3.0
Fatigue	79.0 ± 4.0
Morning tiredness	86.0 ± 5.0
Stiffness	78.0 ± 6.0
Anxiety	46.0 ± 10.0
Depression	38.0 ± 10.0

* All FIQ items were standardized and were scored on a scale of 0–100, where 0 = most healthy state and 100 = most severe state. Higher scores for pain, fatigue, morning tiredness, and stiffness did not affect physical function, but did have a slight effect on job ability.

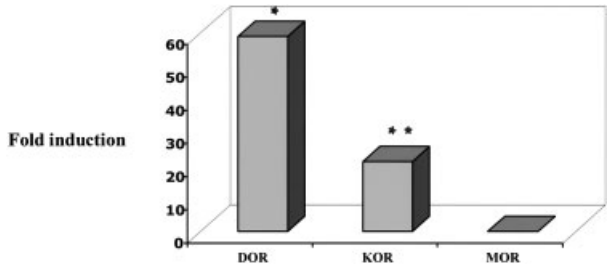


Figure 1. Relative levels of δ-opioid receptor (DOR), κ-opioid receptor (KOR), and μ-opioid receptor (MOR) expression in the skin of fibromyalgia (FM) patients compared with normal controls. The y-axis shows the mean fold increase in expression levels in FM patients. ∗ = $P = 0.001$; ∗∗ = $P = 0.009$, versus normal skin.

detectable at 25 cycles in normal brain tissue but was not dectable in any FM skin or normal skin samples.

In earlier investigations, we showed that the quantities of OR mRNA in muscle did not differ between healthy subjects and FM patients (8). In the present investigation all skin tissue samples examined expressed detectable levels of DOR and KOR genes, with an increased level of expression in FM patients compared with healthy controls.

Although it is believed that DOR, KOR, and MOR are expressed in the CNS, the presence of these receptors in peripheral blood lymphocytes and human lymphoid cell lines has also been reported by a few groups (9,13,14), and we previously demonstrated that they were present in normal skin tissue (10). This is the first study to demonstrate that DOR and KOR are also widely expressed outside the CNS in FM patients, in skin tissue. These results confirm our hypothesis that the expression of ORs differs in the skin of FM patients compared with healthy individuals, probably due to the presence of persistent pain. We did not detect MOR in any skin samples, which is consistent with the findings reported by Wick et al (13) who also demonstrated the absence of MOR in peripheral blood lymphocytes.

The enhanced up-regulation of KOR and DOR in the skin must be taken into consideration with regard to future treatment strategies in FM that use selective opioid agonists. Evidence indicates that the morphine-preferring MOR is the major site for the analgesic action of most opiate drugs. Given our findings, we suggest that DOR and KOR be considered in this context, e.g., as topical application.

Dr. Salemi's work was supported by the Zurzach and Olga-Mayenfisch Foundation. Dr. Sprott's work was supported by the American Fibromyalgia Syndrome Association. Dr. Sprott has received consulting fees (less than $10,000 each) from Zambon Svizzera, MSD, Pfizer, Grünenthal, Janssen-Cilag, and Mundipharma AG.

Dr. Sprott 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. Aeschlimann, Michel, Steffen Gay, Sprott.
Acquisition of data. Salemi, Aeschlimann, Wollina, Sprott.
Analysis and interpretation of data. Salemi, Wollina, Renate E. Gay, Steffen Gay, Sprott.
Manuscript preparation. Salemi, Wollina, Michel, Sprott.
Statistical analysis. Salemi, Sprott.
Organization of experiments and infrastructure. Renate E. Gay.

Souzan Salemi, PhD
University Hospital and University of Zurich
Zurich, Switzerland
Andre Aeschlimann, MD
RehaClinic Zurzach
Zurzach, Switzerland
Uwe Wollina, MD
Stadtisches Klinikum
Dresden, Germany
Renate E. Gay, MD
Beat A. Michel, MD
Steffen Gay, MD
Haiko Sprott, MD
University Hospital and University of Zurich
Zurich, Switzerland

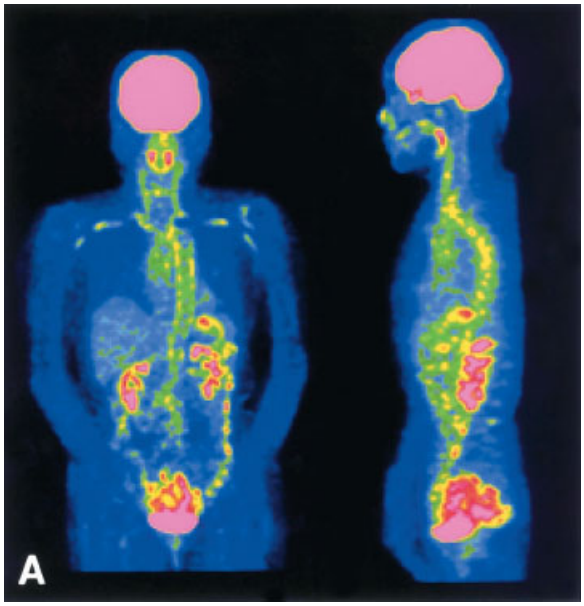
1. Kieffer BL. Opioids: first lessons from knockout mice. Trends Pharmacol Sci 1999;20:19–26.
2. Simonin F, Gaveriaux Ruff C, Befort K, Matthes H, Lannes, B, Micheletti G, et al. κ-opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system. Proc Natl Acad Sci U S A 1995;92:7006–10.
3. Bzdega T, Chin H, Kim H, Jung HH, Kozak CA, Klee WA. Regional expression and chromosomal localization of the δ opiate receptor gene. Proc Natl Acad Sci U S A 1993;90:9305–9.
4. Day DP, Taylor L, Akil A. Prodynorphin biosynthesis and post-translational processing. In: Herz A, editor. Opioids I: handbook of experimental pharmacology. Berlin: Springer; 1993. p. 449–63.
5. Rossier J. Biosynthesis of enkephalin and proenkephalin-derived peptides. In: Herz A, editor. Opioids I: handbook of experimental pharmacology. Berlin: Springer; 1993. p. 423–47.
6. Mason P. Central mechanisms of pain modulation. Curr Opin Neurobiol 1999;9:436–41.
7. May CN, Dashwood MR, Whitehead CJ, Mathias CJ. Differential cardiovascular and respiratory responses to central administration of selective opioid agonists in conscious rabbits: correlation with receptor distribution. Br J Pharmacol 1989;98:903–13.
8. Salemi S, Aeschlimann A, Gay RE, Michel BA, Gay S, Kaeser L, et al. Expression and localization of opioid receptors in muscle satellite cells: no difference between fibromyalgia patients and healthy subjects. Arthritis Rheum 2003;48:3291–3.
9. Shen H, Aeschlimann A, Reisch N, Gay RE, Simmen BR, Michel BA, et al. Kappa and delta opioid receptors are expressed but down-regulated in fibroblast-like synoviocytes of patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 2005;52:1402–10.
10. Salemi S, Aeschlimann A, Reisch N, Jungel A, Gay RE, Heppner FL, et al. Detection of κ and δ opioid receptors in skin outside the nervous system. Biochem Biophys Res Commun 2005;338:1012–7.
11. Burckardt CS, Clark SR, Bennett RM. The Fibromyalgia Impact Questionnaire development and validation. J Rheumatol 1991;18:728–33.
12. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. Arthritis Rheum 1990;33:160–72.
13. Wick MJ, Minnerath SR, Roy S, Ramakrishnan S, Loh HH. Differential expression of opioid receptor genes in human lym-

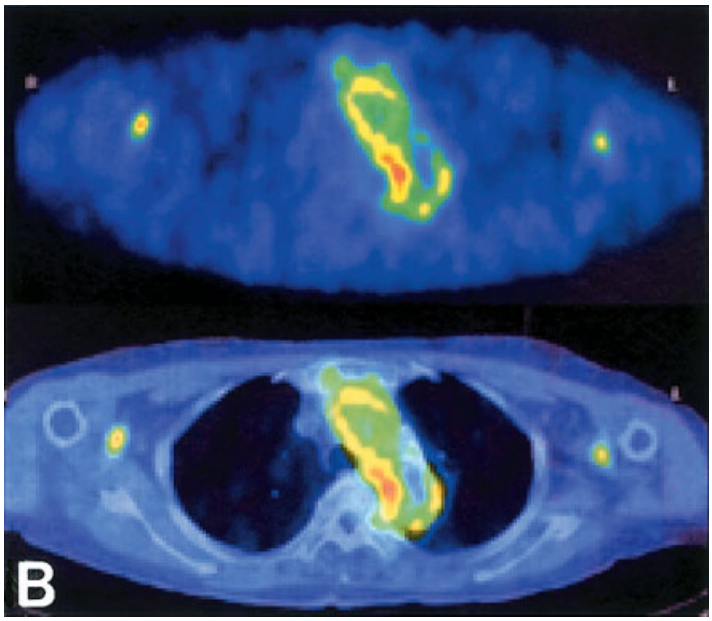
phoid cell lines and peripheral blood lymphocytes. J Neuroimmunol 1996;64:29–36.

14. Cabot PJ. Immune-derived opioids and peripheral antinociception. Clin Exp Pharmacol Physiol 2001;28:230–2.

DOI 10.1002/art.22723

Clinical Images: Takayasu arteritis diagnosed by positron emission tomography





The patient, a 67-year-old woman with no obvious symptoms, presented at our clinic when she experienced a minor injury. Blood tests revealed anemia, an elevated erythrocyte sedimentation rate, and a slightly increased C-reactive protein level. Positron emission tomography (PET) showed positive uptake at the aortic arch, thoracoabdominal aorta, and brachiocephalic artery (**A** and **B**). Angiographic imaging revealed irregularity of the arterial wall at the location identified by PET, and lung perfusion scintigraphy showed defects at the left middle and lower lung fields. Since there were no symptoms suggesting giant cell (temporal) arteritis and no complications or serologic findings indicating arteriosclerosis, Takayasu arteritis was diagnosed. Treatment with high-dose steroids led to pronounced improvement of the lung perfusion and arterial wall thickening within 2 months. To our knowledge, this is the first report of asymptomatic Takayasu arteritis diagnosed by PET, showing the efficacy of this technique in detecting arteritis.

Kunihiro Yamaoka, MD, PhD
Kazuyoshi Saito, MD, PhD
Shingo Nakayamada, MD, PhD
Makiko Yamamoto, MD
Yoshiya Tanaka, MD, PhD
First Department of Internal Medicine
University of Occupational and Environmental Health, Japan
Kitakyushu, Japan