0022-5347/01/1665-1738/0
THE JOURNAL OF UROLOGY®

Vol. 166, 1738–1741, November 2001
Printed in U.S.A.

CORRELATION OF β-ENDORPHIN AND PROSTAGLANDIN E2 LEVELS IN PROSTATIC FLUID OF PATIENTS WITH CHRONIC PROSTATITIS WITH DIAGNOSIS AND TREATMENT RESPONSE

ASHA R. SHAHED AND DANIEL A. SHOSKES*

Harbor-University of California-Los Angeles Medical Center, Torrance, California, and Cleveland Clinic Florida, Fort Lauderdale, Florida

ABSTRACT

Purpose: The chronic pelvic pain syndrome is a clinically defined symptom complex of unclear etiology. We have noted increased oxidative stress in the prostatic fluid of these patients, implying an active inflammatory response. Immune cells can produce the natural opioid β-endorphin at the site of injury, which may modulate pain. We measured β-endorphin and the inflammatory marker prostaglandin E2 in the expressed prostatic secretions of men with prostatitis, and correlated the results with symptoms.

Materials and Methods: Expressed prostatic secretions samples from 70 patients and 8 asymptomatic controls were collected and frozen. β-Endorphin and prostaglandin E2 were measured by enzyme-linked immunosorbent assay. Results were stratified according to prostatitis category and compared in individuals before and after therapy.

Results: In symptomatic patients β-endorphin and prostaglandin E2 were not significantly different in categories II, IIIa and IIIb expressed prostatic secretions but they were higher than in controls. The mean β-endorphin level plus or minus standard error of mean in symptomatic patients was significantly higher (23.8 ± 11 ng./ml. versus 8.7 ± 4.7, $p = 0.0001$) and mean prostaglandin E2 was lower (6.01 ± 2.9 ng./ml. versus 3.01 ± 2.9, $p = 0.001$) after successful therapy with antibiotics or antioxidant phytotherapy, Prosta-Q (Farr Laboratories, Santa Clarita, California).

Conclusions: We observed a correlation of higher prostaglandin E2 and lower β-endorphin in symptomatic men with chronic prostatitis. Increased oxidative stress and inflammation may induce prostaglandin E2 production that would inhibit β-endorphin release. Treatment with therapeutic agents that decrease oxidative stress, such as antibiotics and antioxidant phytotherapy, may function at least partially by increasing β-endorphin and decreasing prostaglandin E2.

KEY WORDS: prostate, prostatitis, endorphins, quercetin

The chronic pelvic pain syndrome is a common urological condition with a controversial etiology. While many patients have bacterial growth in the prostate, to our knowledge the pathogenic role of these bacteria, especially those considered nonuropathogens, is unproved and antibiotic therapy is not always effective. There are many alternate theories of etiology including autoimmune, neuromuscular and chemical causes. Recently we have shown that the biochemical and molecular markers of oxidative stress are elevated in the expressed prostatic secretions of men with National Institutes of Health categories II and III prostatitis compared with controls and successful therapy reverses this elevation.[1,2]

The role of prostaglandins in inflammation, pain perception and chronic inflammatory diseases is widely recognized. Prostaglandins such as prostaglandin E2 are the products of arachidonic acid metabolism catalyzed by cyclooxygenase. Cyclooxygenase-1, 1 of the 2 isoforms of cyclooxygenase, is expressed constitutively. Cyclooxygenase-2 is inducible by oxidative stress, inflammation[3] growth factors, carcinogens and tumor promotors.[4] Cyclooxygenase-2 is thought to be a proinflammatory enzyme[3] and specific inhibitors are used as anti-inflammatory and analgesic agents to treat various diseases, including the chronic pelvic pain syndrome. Others have reported that the concentration of prostaglandin E2 was higher in the expressed prostatic secretions of men with prostatitis than in controls.[5]

Opioids, which are produced by the central nervous system, have a significant role in pain reduction. Opioids and especially endorphins have also been shown to be produced by immune cells at the site of injury or inflammation.[6–12] Three families of opioids that are derived from a distinct gene are products of precursor proteins. Proteolytic processing of the large precursor proteins proopimelanocortin, proenkephlin and prodynorphin results in β-endorphin, enkephlin and dynorphin, respectively. These peptides bind to μ, δ or κ receptors with different affinities and specificities. Opioid receptors have been identified in peripheral tissue and proopimelanocortin messenger (m)RNA expression has been observed in immune cells at the site of inflammation.[12] β-Endorphin has also been detected in the seminal plasma of patients with some urological diseases.[13] Thus, strong evidence implies that immune cells, especially lymphocytes and monocytes, produce β-endorphin at the site of inflammation to decrease pain. These findings open the path to novel approaches to pain caused by inflammatory diseases.

Prostaglandin E2 is known to inhibit β-endorphin release in animal models.[14] In surgical patients plasma β-endorphin was higher in those pretreated with a nonsteroidal anti-inflammatory than in those not treated, indicating that this

Accepted for publication June 15, 2001.
Supported by National Institutes of Health Grant DK53738-04 and the Prostatitis Foundation.
* Financial interest and/or other relationship with Boehringer Ingelheim, Triurol, Merck, Bristol-Myers-Squibb, Argomed and Roche.

drug may act by lowering prostaglandin E2.[15] Therefore, prostaglandin E2 and β-endorphin may be related in the regulation and perception of inflammation and pain.

We hypothesized that increased oxidative stress may upregulate cyclooxygenase-2, and oxidative stress and/or cyclooxygenase-2 expression may interfere with the ability of immune cells to produce β-endorphin, resulting in pain in prostatitis. To test this hypothesis we measured the level of prostaglandin E2 and β-endorphin in the expressed prostatic secretions of men with prostatitis and asymptomatic controls by enzyme-linked immunosorbent assay (ELISA). We then correlated the results with symptoms before and after therapy.

MATERIALS AND METHODS

Samples of expressed prostatic secretions were obtained from 70 symptomatic patients at the Harbor-University of California-Los Angeles Medical Center Chronic Prostatitis Clinic. In 35 cases samples were also available after therapy. All patients had had chronic pelvic pain for at least 3 of the previous 6 months without an active urinary tract infection. In addition, 8 men with no clinical evidence of chronic prostatitis or the chronic pelvic pain syndrome consented to prostatic massage and use of their expressed prostatic secretions for research, including 4 with benign prostatic hyperplasia, 1 subsequently diagnosed with prostate cancer and 3 with no urinary or prostatic symptoms. The samples were placed into cryovials and stored at −80C until use.

All samples from the urethra, mid stream urine, expressed prostatic secretions and post massage urine were cultured using standard laboratory technique for aerobic bacteria for 5 days and any growth was reported. Patients with bacterial growth in expressed prostatic secretions or post-massage urine that was unique to these samples of at least 100-fold greater than counts in urethra or mid stream urine specimens were classified with category II disease. The white blood count in the expressed prostatic secretions was estimated using a wet mount under 400× total magnification and reported as the mean of 4 fields. Patients with negative cultures were classified with category IIIa disease when there were 10 or greater white blood cells per high power field and IIIb when there were fewer white blood cells.

Successful therapy was defined as significant improvement in patient symptoms according to patient satisfaction, which did not require any new or additional therapy. Of the few men for whom pretreatment and posttreatment National Institutes of Health-Chronic Prostatitis Symptom Index scores were available those who were satisfied had score improvement of at least 25%, a finding that we have reported in a previous study.[2]

Category II disease was managed by antibiotics based on culture sensitivity and the patient response to previous therapy. Most patients received a quinolone or macrolide antibiotic for a minimum of 4 and a maximum of 8 weeks. Category III disease was managed by antibiotics in men without previous adequate antibiotic therapy or by 500 mg. of the herbal supplement quercetin in capsule form. The latter patients received 1 capsule twice daily for 4 weeks.

Samples of expressed prostatic secretions were centrifuged at 4,000 rpm for 5 minutes and duplicate 5 to 10 μl. samples of supernatant were used to measure prostaglandin E2 using an ELISA kit (Caymen Chemical Co., Ann Arbor, Michigan) according to manufacturer directions. A standard prostaglandin E2 curve run on the same plate was used for calculations. Prostaglandin E2 was not extracted from expressed prostatic secretions due to small sample volume but results were verified by measuring several dilutions. A 2.5 to 5 μl. specimen of expressed prostatic secretions was used for β-endorphin measurement with an ELISA kit (Peninsula Laboratories, San Carlos, California) according to manufacturer directions. Results are presented in ng./ml. of expressed prostatic secretions.

We performed reverse transcriptase-polymerase chain reaction (RT-PCR) of proopimelanocortin and cyclooxygenase-2 mRNA expression. Total RNA and complementary DNA synthesis in the expressed prostatic secretions was done as previously described.[1] PCR was performed using standard methods with human proopimelanocortin[16] and cyclooxygenase-2 primers.[17]

Data presented as the mean plus or minus standard error were analyzed using single factor analysis of variance. When analysis of variance was significant, individual comparisons were made using the unpaired Student t test. Comparisons were made before and after treatment using the paired t test with significance considered at $p < 0.05$.

RESULTS

Based on culture results and the white blood count, disease was category II in 22 men, category IIIa in 28 and category IIIb in 20. Mean prostaglandin E2 and β-endorphin in the expressed prostatic secretions of the 8 asymptomatic controls were 0.94 ± 0.6 ng./ml. and 3.29 ± 2.36, respectively. Prostaglandin E2 and β-endorphin in the expressed prostatic secretions of patients with category II, IIIa and IIIb prostatitis were not significantly different among categories but they were different than control values (fig. 1). Figure 2 shows the level of prostaglandin E2 and β-endorphin in the expressed prostatic secretions of 35 men with complaints of pain and discomfort, and after successful treatment. The average β-endorphin level increased 2.75-fold and prostaglandin E2 decreased by 50% in patients with improved symptoms compared with those with a higher level of discomfort. Of these 35 men for whom pretreatment and posttreatment expressed prostatic secretions were available for assay 20 had category II, 9 had category IIIa and 6 had category IIIb disease. Those with positive cultures (category II) were treated with antibiotics, usually quinolones or macrolides. Some category III cases were managed by antibiotics and the remainder were managed by the bioflavonoid antioxidant Prosta-Q. In 6 of the 7 latter men β-endorphin increased and prostaglandin E2 decreased substantially compared with pretreatment levels (fig. 3).

In 21 samples of expressed prostatic secretions with extra volume when possible, we measured the expression of proopimelanocortin and cyclooxygenase-2 mRNA by RT-PCR. Little to no measurable expression of either type of mRNA was identified in the expressed prostatic secretions of asymptomatic controls. In the 10 samples in which we measured prostaglandin E2 and β-endorphin plus proopimelanocortin and cyclooxygenase-2 by RT-PCR higher prostaglandin E2 and β-endorphin corresponded with measurable gene expression of cyclooxygenase-2 and proopimelanocortin, respectively.

DISCUSSION

Pain is the most common symptom of chronic prostatitis and it is observed in categories II and III. Whether the cause is infection, inflammation or neuromuscular spasm, all cases involve symptoms that make them clinically indistinguishable. The common factors are pain and evidence of an inflammatory response. Even when white blood cells are not identified in expressed prostatic secretions or on prostate biopsy, there is evidence for elevated cytokines and elevated oxidative stress.[1] The cyclooxygenase-2 pathway is induced by oxidative stress, which may result in pain due to prostaglandin production. In our series prostaglandin E2 in patients with prostatitis were about 4- to 6-fold higher than in asymptomatic controls. High prostaglandin E2 in the expressed prostatic secretions of men with prostatitis has previously been reported.[5] Interestingly there was no difference in categories II, IIIa and IIIb cases in terms of prostaglandin E2,

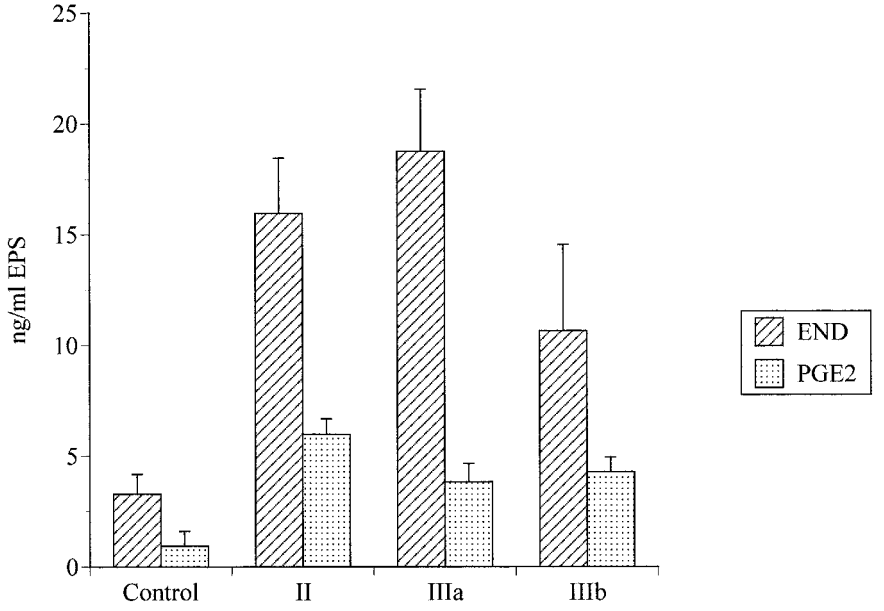

FIG. 1. Mean β-endorphin (*END*) and prostaglandin E2 (*PGE2*) plus or minus SEM in 5 to 10 μl. of expressed prostatic secretions (*EPS*) of 70 patients with prostatitis, including 22 with category II, 28 category IIIa and 20 category IIIb disease.

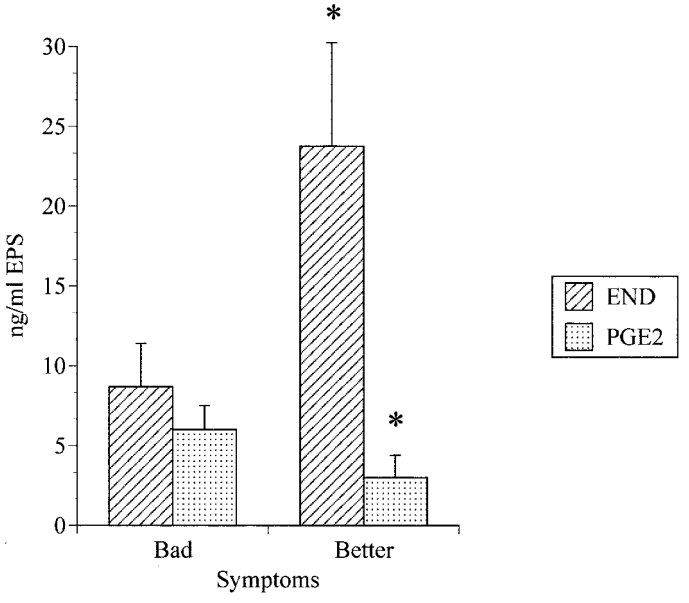

FIG. 2. Mean β-endorphin (*END*) and prostaglandin E2 (*PGE2*) plus or minus SEM in 5 to 10 μl. of expressed prostatic secretions (*EPS*) of 35 patients with prostatitis before and after treatment. Each group contained same patients before and after therapy. Asterisk indicates $p < 0.001$.

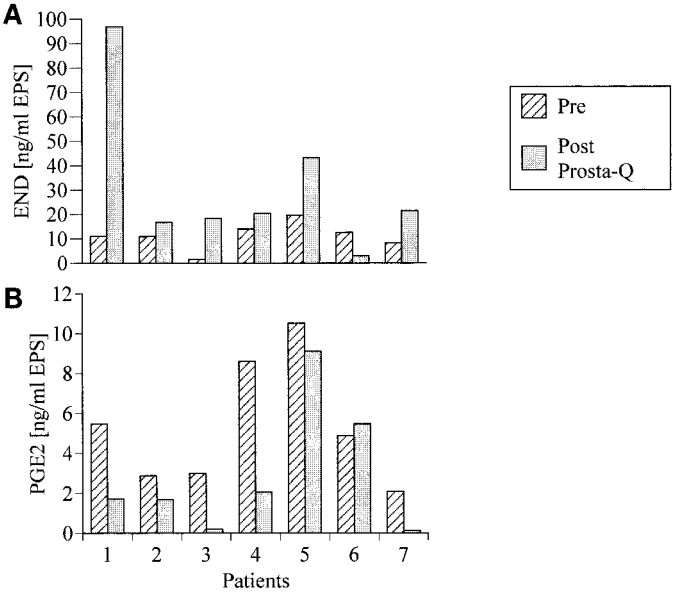

FIG. 3. β-Endorphin (*END*) and prostaglandin E2 (*PGE2*) in 5 to 10 μl. of expressed prostatic secretions (*EPS*) of patients before (*Pre*) and after (*Post*) Prosta-Q.

although a trend to lower levels was evident in category IIIb. This finding implies that despite potentially diverse etiologies there may be common final pathways of pain.

We compared the levels of prostaglandin E2 and β-endorphin in the expressed prostatic secretions of patients for whom pretreatment and posttreatment samples were available and noted an inverse relationship between the 2 values. We propose that inflammatory cells in the prostate of men with prostatitis can produce β-endorphin locally and this ability may be inhibited by high prostaglandin E2. The inhibition of β-endorphin release by prostaglandin E2 has been reported for pituitary corticotroph cells.[14] In addition, β-endorphin release in pituitary cell cultures was inhibited by adding of prostaglandin E2 and increased by cyclooxygenase-2 inhibitors.[13] In preliminary studies proopimelanocortin and cyclooxygenase-2 mRNA expression in cells isolated from expressed prostatic secretions correlated with higher β-endorphin and prostaglandin E2, indicating that inflammatory cells in the prostate may produce β-endorphin locally. Therefore, effective therapy for chronic prostatitis may resolve symptoms by decreasing the level of prostaglandin that has been acting to inhibit β-endorphin release.

In patients with a urinary tract infection sterilization with antibiotics may stop the inflammatory response, leading to decreased prostaglandin E2. In those without a urinary tract infection treatment with bioflavonoid antioxidants may be directly effective on the inflammatory cells, resulting in the same response. The bioflavonoid preparation Prosta-Q has significantly improved symptoms in 82% of men with category III prostatitis[2] and has decreased oxidative stress in the expressed prostatic secretions.[1] The bioflavonoid curcumin

has inhibited cyclooxygenase-2 transcription in gastrointestinal epithelial cells.[18] We suggest that bioflavonoids may be effective as antioxidants and perhaps also by lowering prostaglandin E2, thereby, increasing β-endorphin release.

CONCLUSIONS

We observed elevated prostaglandin E2 and decreased β-endorphin in the expressed prostatic secretions of men with symptomatic chronic prostatitis. Therapy with antibiotics or antioxidant phytotherapy decreased prostaglandin E2 and increased β-endorphin. Pain in prostatitis may be due to increased prostaglandin production, which may then inhibit local β-endorphin production. These findings may potentially lead to novel therapy for prostatitis that specifically targets these pathways.

REFERENCES

1. Shahed, A. R. and Shoskes, D. A.: Oxidative stress in prostatic fluid of patients with chronic pelvic pain syndrome: correlation with gram positive bacterial growth and treatment response. J Androl, **21:** 669, 2000
2. Shoskes, D. A., Zeitlin, S. I., Shahed, A. et al: Quercetin in men with category III chronic prostatitis: a preliminary prospective, double-blinded, placebo controlled trial. Urology, **54:** 960, 1999
3. Gilroy, D. W., Colville-Nash, P. R., Willis, D. et al: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med, **5:** 698, 1999
4. Subbaramaiah, K., Telang, N., Ramonetti, J. T. et al: Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res, **56:** 4424, 1996
5. Bauer, H. W. and Bach, D.: Prostaglandin E2 in prostatitis and prostatic adenoma. Urol Int, **41:** 139, 1986
6. Schafer, M., Carter, L. and Stein, C.: Interleukin 1 beta and corticotropin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue. Proc Natl Acad Sci USA, **91:** 4219, 1994
7. Machelska, H., Cabot, P. J., Mousa, S. A. et al: Pain control in inflammation governed by selectins. Nat Med, **4:** 1425, 1998
8. Cabot, P. J., Carter, L., Gaiddon, C. et al: Immune cell-derived beta-endorphin. Production, release and control of inflammatory pain in rats. J Clin Invest, **100:** 142, 1997
9. Corsi, M. M., Fulgenzi, A., Tiengo, M. et al: Effect of somatostatin on beta-endorphin release in rat experimental chronic inflammation. Life Sci, **64:** 2247, 1999
10. Stein, C., Gramsch, C. and Herz, A.: Intrinsic mechanisms of antinociception in inflammation: local opioid receptors and beta-endorphin. J Neurosci, **10:** 1292, 1990
11. Porreca, F., Lai, J. and Malan, T. P., Jr.: Can inflammation relieve pain? Nat Med, **4:** 1359, 1998
12. Panerai, A. E. and Sacerdote, P.: Beta-endorphin in the immune system: a role at last? Immunol Today, **18:** 317, 1997
13. Zalata, A., Hafez, T., Van Hoecke, M. J. et al: Evaluation of beta-endorphin and interleukin-6 in seminal plasma of patients with certain andrological diseases. Hum Reprod, **10:** 3161, 1995
14. Vlaskovska, M., Hertting, G. and Knepel, W.: Adrenocorticotropin and beta-endorphin release from rat adenohypophysis in vitro: inhibition by prostaglandin E2 formed locally in response to vasopressin and corticotropin-releasing factor. Endocrinology, **115:** 895, 1984
15. Troullos, E., Hargreaves, K. M. and Dionne, R. A.: Ibuprofen elevates immunoreactive beta-endorphin levels in humans during surgical stress. Clin Pharmacol Ther, **62:** 74, 1997
16. Slominsky, A., Heasley, D., Mazurkiewicz, J. E. et al: Expression of proopipmelanocortin (POMC)-derived melanocyte-stimulating hormone and adrenocorticotropic hormone (ACTH) peptides in skin of basal cell carcinoma patients. Hum Pathol, **30:** 208, 1999
17. Hla, T. and Neilson, K.: Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci USA, **89:** 7384, 1992
18. Zhang, F., Altorki, N. K., Mestre, J. R. et al: Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated gastrointestinal epithelial cells. Carcinogenesis, **20:** 445, 1999