ARTHRITIS & RHEUMATISM
Vol. 42, No. 12, December 1999, pp 2561–2568

FLUOXETINE AND AMITRIPTYLINE INHIBIT NITRIC OXIDE, PROSTAGLANDIN E_2, AND HYALURONIC ACID PRODUCTION IN HUMAN SYNOVIAL CELLS AND SYNOVIAL TISSUE CULTURES

ILANA YARON, IDIT SHIRAZI, RACHEL JUDOVICH, DAVID LEVARTOVSKY, DAN CASPI, and MICHAEL YARON

Objective. **To evaluate the effects of fluoxetine and amitriptyline on nitric oxide (NO), prostaglandin E_2 (PGE_2), and hyaluronic acid (HA) production in human synovial cells and synovial tissue cultures.**

Methods. **Human synovial cells, synovial tissue, and cartilage were cultured in the presence or absence of cytokines, lipopolysaccharides (LPS), fluoxetine, or amitriptyline. Production of NO, PGE_2, and HA was determined in culture media. Sulfated glycosaminoglycan (S-GAG) synthesis was evaluated in cartilage by ^{35}S incorporation.**

Results. **Fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) inhibited NO release by 56%, 62%, and 71%, respectively, in the media of synovial cells stimulated by interleukin-1α (IL-1α; 1 ng/ml) plus tumor necrosis factor α (TNFα; 30 ng/ml). Amitriptyline (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) caused a 16%, 27.3%, and 51.4% inhibition of NO release. Fluoxetine and amitriptyline (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) significantly ($P < 0.05$) inhibited PGE_2 release in the media of human synovial cells in the presence of IL-1α plus TNFα, in a dose-dependent manner (up to 88% inhibition). Fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) and amitriptyline (1 μg/ml and 3 μg/ml) significantly ($P < 0.05$) inhibited PGE_2 release in the media of human synovial tissue in the presence of LPS. Fluoxetine and amitriptyline (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) also significantly ($P < 0.05$) inhibited HA production by human synovial cells in the presence of IL-1β plus TNFα. Fluoxetine and amitriptyline (1 μg/ml) partially reversed IL-1β–induced inhibition of ^{35}S-GAG synthesis by human cartilage cultures ($P < 0.05$). Neither fluoxetine nor amitriptyline had a toxic effect on cells in the concentrations used.**

Conclusion. **Inhibition of NO and PGE_2 production by connective tissue cells is a mechanism by which some antidepressant medications may affect pain, articular inflammation, and joint damage.**

Antidepressants are being used as a supplementary therapy in both inflammatory and noninflammatory chronic rheumatic diseases. Amitriptyline and, more recently, fluoxetine have been used in the management of fibromyalgia (1,2). Furthermore, they are used in the management of osteoarthritis (OA) and rheumatoid arthritis (RA) to combat secondary depression resulting from these chronic illnesses (3). An inhibitory effect on experimental animal inflammation has been described for tricyclic antidepressants (4) and, more recently, for fluoxetine as well (5). The mechanism of their inhibitory effect on experimental animal inflammation is not clear, nor is their mode of action in patients with fibromyalgia.

Prostaglandin E_2 (PGE_2) and nitric oxide (NO) mediate inflammation and pain (6–8). Overproduction of hyaluronic acid (HA) is an integral part of connective tissue cell activation during inflammation (9). Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) play important roles in the pathogenesis of OA (10) and RA (11), respectively. Lipopolysaccharides (LPS) are inducers of IL-1, TNFα, NO, and PGE_2 in different types of cells (12–14). We report herein the effects of amitriptyline and fluoxetine on the production of NO, PGE_2, and HA in human synovial cells and synovial tissue cultures, and on the synthesis of sulfated glycosaminoglycan (S-GAG) in cartilage cultures, in the presence of either IL-1(α or β) and TNFα or LPS.

Supported by the Research Fund, Ichilov Hospital.
Ilana Yaron, MSc, Idit Shirazi, PhD, Rachel Judovich, MSc, David Levartovsky, MD, Dan Caspi, MD, Michael Yaron, MD: Ichilov Hospital, Tel-Aviv Sourasky Medical Center, and Tel Aviv University, Tel Aviv, Israel.

Address reprint requests to Michael Yaron, MD, Department of Rheumatology, Ichilov Hospital, 6 Weizmann Street, Tel Aviv 64239, Israel.
Submitted for publication November 12, 1998; accepted in revised form July 2, 1999.

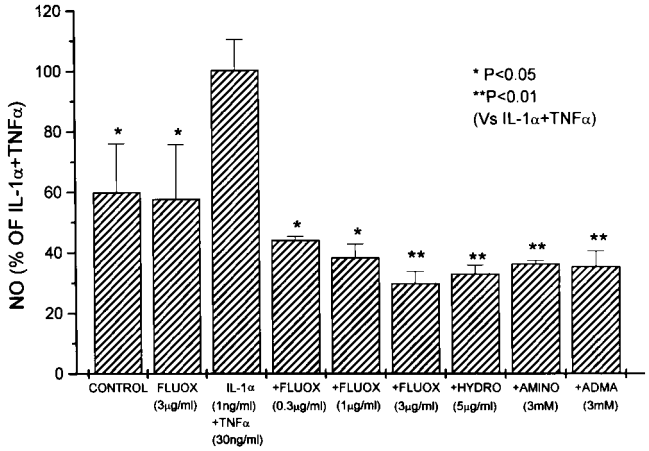

Figure 1. Effect of fluoxetine (FLUOX; 0.3 μg/ml, 1 μg/ml, and 3 μg/ml), hydrocortisone (HYDRO; 5 μg/ml), aminoguanidine (AMINO; 3 m*M*), and asymmetric dimethylarginine (ADMA; 3 m*M*) on the release of nitric oxide (NO) in the media of synovial cell cultures stimulated by interleukin-1α (IL-1α; 1 ng/ml) plus tumor necrosis factor α (TNFα; 30 ng/ml). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM level of NO in control cultures was 71 ± 19 nmoles/10^6 cells and that in IL-1α plus TNFα–stimulated cultures was 118 ± 13 nmoles/10^6 cells.

MATERIALS AND METHODS

IL-1α, IL-1β, and TNFα were from Biogen SA (Geneva, Switzerland). LPS was from Difco Laboratories (Detroit, MI). IL-1α or IL-1β was used at random according to their technical availability at the time that the experiments were performed, since previous experiments showed very similar effects of IL-1α and IL-1β in our model. Amitriptyline, diazepam, nialamide, aminoguanidine, and asymmetric dimethylarginine (ADMA) were from Sigma (St. Louis, MO). Fluoxetine pure substance was a donation from Eli Lilly (Indianapolis, IN). Nitroprusside was from Schwarz Pharma (Mannheim, Germany). Cytokines or LPS and test materials were concomitantly added to cultures.

Specimen selection and culture conditions. Synovial tissue and cartilage were obtained during surgery from 16 patients who were undergoing total knee or hip replacement for OA (4 men, mean ± SD age 71.6 ± 0.58 years, 12 women, age 71.4 ± 2 years) and from 1 female patient with RA (age 71) who was undergoing total knee replacement.

Synovial cells derived from small pieces (2 mm in diameter) of human synovial tissue were grown after trypsinization in monolayers in tissue culture flasks, by methods described by Castor et al (9). Synovial cell lines were thus obtained and experiments performed in passages 2–4 (after first trypsinization). The culture medium consisted of RPMI 1640, supplemented with L-glutamine (2 m*M*), penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml), and 10% fetal calf serum (FCS; Biological Industries, Beit Haemek, Israel). Experiments were performed in 96-well plates containing 15–20 × 10^3 cells per well in 1% FCS. Incubation time (37°C, 5% CO_2) with different additives was 48–72 hours.

Organ cultures. Within 2 hours of removal, synovial tissues were cut into small pieces (a few mm in diameter) and cultured in 2-cm Petri dishes with test materials in RPMI 1640 supplemented with L-glutamine (2 m*M*), penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml). Following 48 hours of incubation (37°C, 5% CO_2), NO, PGE_2, and HA were measured in culture media.

Cartilage tissue was finely diced, randomized, and distributed uniformly in 96-well plates containing 0.2 ml Dulbecco's modified Eagle's medium, 1% FCS, penicillin, and streptomycin. Test materials were added and followed up 24 hours later by carrier-free $^{35}SO_4$ (specific activity 1,100 Ci/mmoles; Amersham, Buckinghamshire, UK) at a final concentration of 40 μCi/ml. After 2 days of further incubation, the supernatants and cartilage were collected for analysis of S-GAG.

NO, PGE_2, and HA determinations. NO synthesis was determined as previously described by Ashab et al (15). NO_2 and NO_3 were determined after the reduction of NO_3 to NO_2 by a 90-minute incubation in a tilting bath (37°C) using nitrate reductase from *Escherichia coli* and beta nicotinamide adenine dinucleotide phosphate (reduced form) (Sigma) as cofactor. The presence of NO_2 was determined with Griess reagent. Sensitivity of the procedure was 3 μM. PGE_2 was determined by radioimmunoassay, and HA by ^{14}C-glucosamine uptake using the cetyl pyridinium chloride fixation wash procedure (16).

^{35}S-GAG determination. Incorporation of the SO_4 radioactive label into GAG was determined by the cetyl pyridinium chloride fixation wash procedure (16). S-GAG levels were determined in proteolytic digests of combined cartilage tissue and culture medium. Digestion was performed at 56°C for 16 hours with pronase (0.5 mg/ml) in 0.05*M* Tris buffer, pH 7.6 (Sigma).

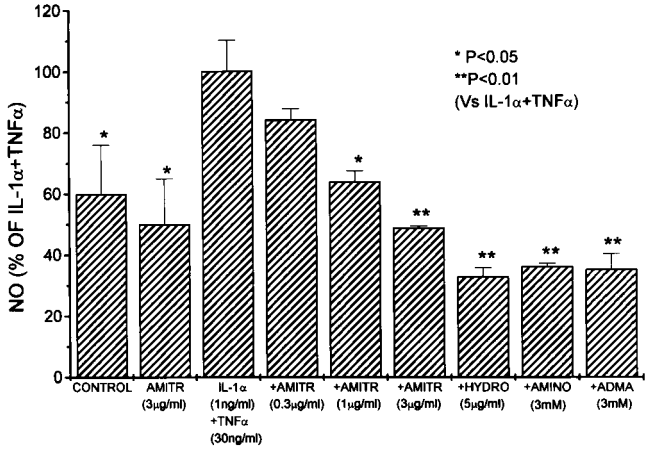

Figure 2. Effect of amitriptyline (AMITR; 0.3 μg/ml, 1 μg/ml, and 3 μg/ml), hydrocortisone (5 μg/ml), aminoguanidine (3 m*M*), and ADMA (3 m*M*) on the release of NO in the media of synovial cell cultures stimulated by IL-1α (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM level of NO in control cultures was 88 ± 11 nmoles/10^6 cells and that in IL-1α plus TNFα–stimulated cultures was 113 ± 11 nmoles/10^6 cells. See Figure 1 for other definitions.

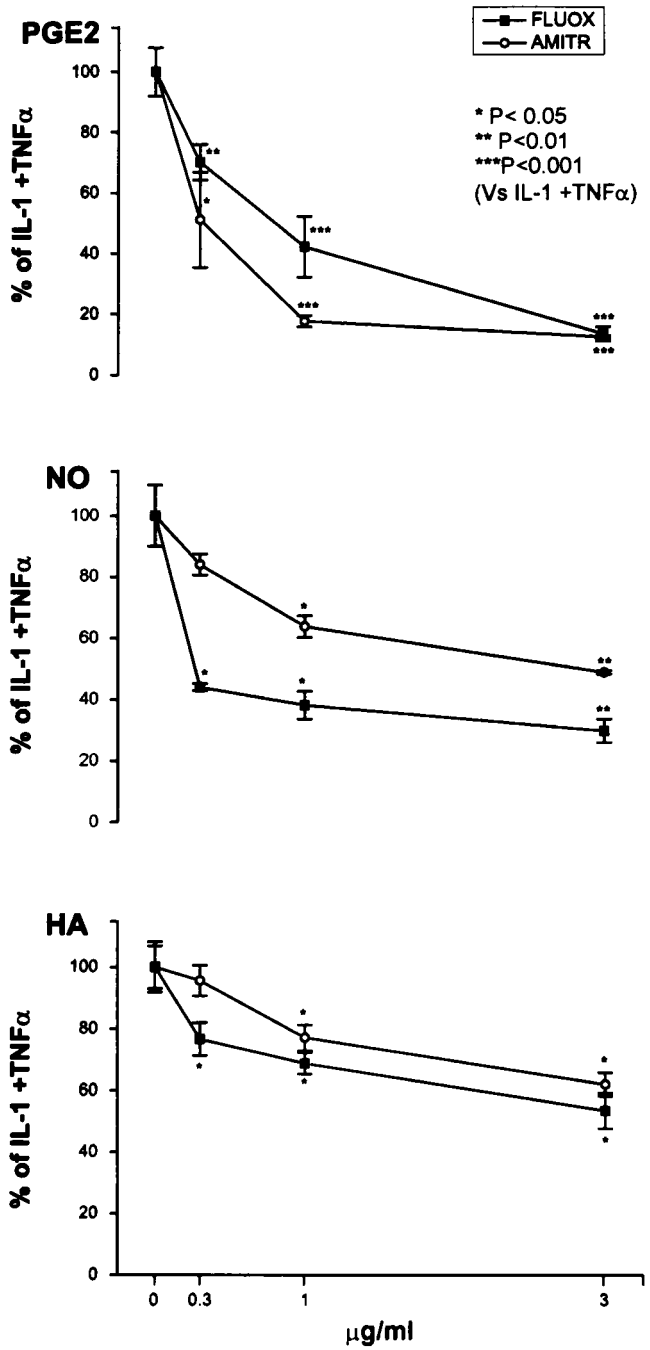

Figure 3. Dose-response curve for the effect of fluoxetine and amitriptyline on the production of prostaglandin E_2 (PGE2), NO, and hyaluronic acid (HA) by human synovial cell cultures stimulated with IL-1 (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean ± SEM. See Figures 1 and 2 for other definitions.

Cell viability and toxicity determinations. Cell viability and toxicity in the presence of amitriptyline or fluoxetine were determined in human synovial fibroblast monolayer cultures by trypan blue exclusion test and by the tetrazolium salt XTT assay (17).

Statistical analysis. In synovial cell cultures, absolute values of NO, PGE_2, and HA were expressed per well (15–20 $\times$ 10^3 cells). In synovial tissue and articular cartilage cultures, determinations were on a per mg tissue basis.

Statistical significance (determination of *P* values) was evaluated by analysis of variance and by Student's *t*-test. *P* values of less than 0.05 were considered statistically significant.

Individual experiments were performed in triplicate or quadruplicate. Results reported as the percentage of stimulated cultures represent the findings from several separate experiments. The number of experiments performed and the absolute values for controls and stimulated cultures are reported in the figure legends.

RESULTS

NO production. Fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) and amitriptyline (1 μg/ml and 3 μg/ml) significantly and dose-dependently inhibited NO release in the media of synovial cells in the presence of IL-1α (1 ng/ml) plus TNFα (30 ng/ml) (Figures 1, 2, and 3). Fluoxetine induced inhibition by 56%, 62%, and 71%, respectively, and amitriptyline by 16%, 27.3%, and 51.4%, respectively, in the 3 dosages administered. Hydrocortisone (5 μg/ml), aminoguanidine (3 m*M*), and ADMA (3 m*M*) also abrogated stimulated production of NO (Figures 1 and 2). The same concentrations of fluoxetine and amitriptyline inhibited, in a similarly

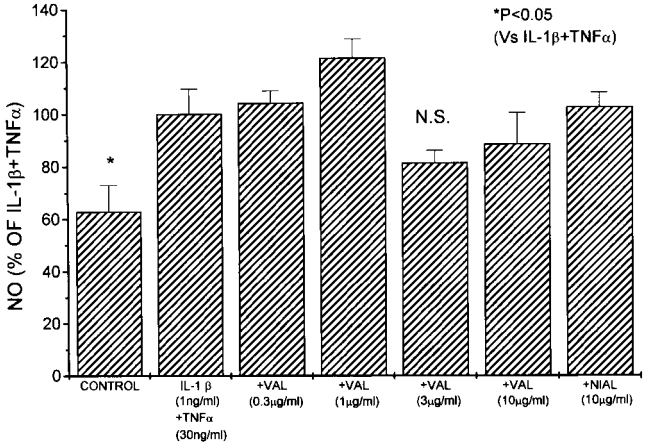

Figure 4. Effect of diazepam (VAL; 0.3 μg/ml, 1 μg/ml, 3 μg/ml, and 10 μg/ml) and that of nialamide (NIAL; 10 μg/ml) on NO production by human synovial cell cultures stimulated with IL-1β (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM level of NO in control cultures was 80 ± 0.8 nmoles/10^6 cells and that in IL-1β plus TNFα–stimulated cultures was 134 ± 10.3 nmoles/10^6 cells. N.S. = not significant (see Figure 1 for other definitions).

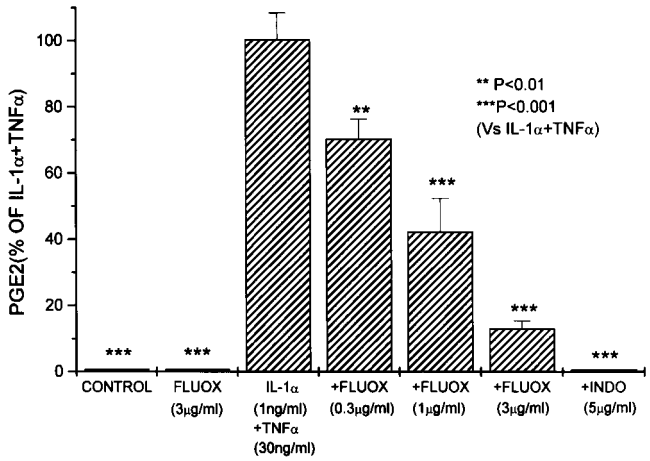

Figure 5. Effect of fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) on the release of prostaglandin E_2 (PGE2) in the media of synovial cell cultures stimulated with IL-1α (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean and SEM of 4 separate experiments (n = 4). The absolute mean ± SEM level of PGE_2 in control cultures was <0.1 ng/well and that in IL-1α plus TNFα–stimulated cultures was 7.4 ± 0.59 ng/well (20,000 cells). INDO = indomethacin (see Figure 1 for other definitions).

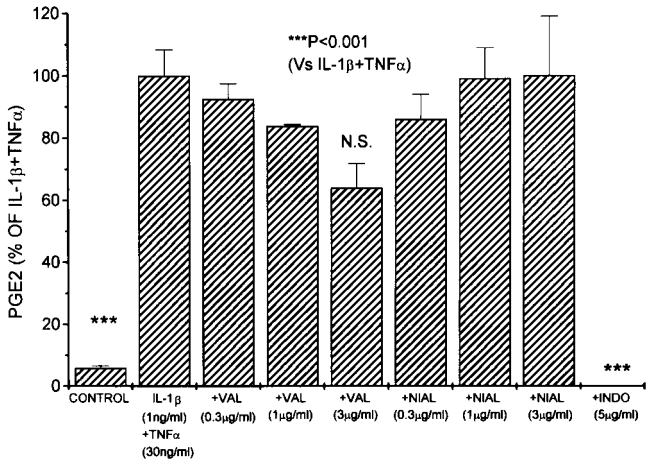

Figure 7. Effect of diazepam (VAL) and nialamide (NIAL) (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) and that of indomethacin (INDO; 5 μg/ml) on prostaglandin E_2 (PGE2) production by human synovial cell cultures stimulated by IL-1β (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM level of PGE_2 in control cultures was <0.1 ng/well (20,000 cells) and that in IL-1β plus TNFα–stimulated cultures was 3.5 ± 0.45 ng/well (20,000 cells). See Figure 1 for other definitions.

significant manner, IL-1–stimulated NO and PGE production in rheumatoid synovial fibroblast cultures (data not shown). NO levels obtained in control cultures (mean ± SEM 71 ± 19 nmoles/10^6 cells) were low and toward the lower end of sensitivity of the Griess reaction assay. Nevertheless, the effects of fluoxetine and amitriptyline on the basal levels of NO were apparently negligible (Figures 1 and 2). Diazepam and nialamide had no significant effect on NO production by stimulated cultures (Figure 4).

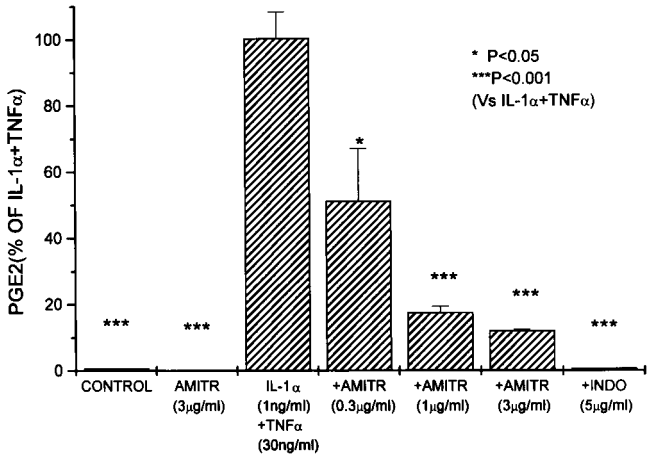

Figure 6. Effect of amitriptyline (AMITR; 0.3 μg/ml, 1 μg/ml, and 3 μg/ml) on the release of prostaglandin E_2 (PGE2) in the media of synovial cell cultures stimulated by IL-1α (1 ng/ml) plus TNFα (30 ng/ml). Bars show the mean and SEM of 4 separate experiments (n = 4). The absolute mean ± SEM level of PGE_2 in control cultures was <0.1 ng/well and that in IL-1α plus TNFα–stimulated cultures was 7.4 ± 0.59 ng/well (20,000 cells). INDO = indomethacin (see Figure 1 for other definitions).

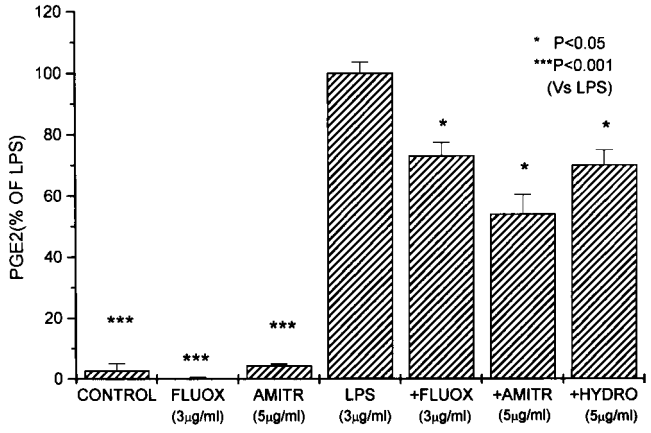

Figure 8. Effect of fluoxetine (FLUOX; 3 μg/ml), amitriptyline (AMITR; 5 μg/ml), and hydrocortisone (HYDRO; 5 μg/ml) on the release of prostaglandin E_2 (PGE2) in the media of synovial tissue cultures stimulated with lipopolysaccharides (LPS; 3 μg/ml). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM level of PGE_2 in control cultures was 1.26 ± 1 ng/mg synovial tissue and that in LPS-stimulated cultures was 42.5 ± 1.48 ng/mg synovial tissue.

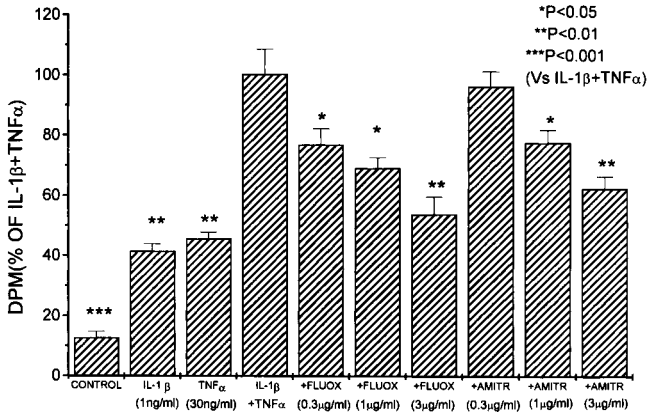

Figure 9. Effect of fluoxetine and amitriptyline (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) on hyaluronic acid production by IL-1β (1 ng/ml) plus TNFα (30 ng/ml)–stimulated synovial cells, as measured by ^{14}C-glycosamine uptake. Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM value in control cultures was 154 ± 25.5 disintegrations per minute (DPM)/well and that in IL-1β plus TNFα–stimulated cultures was 1,207 ± 99 dpm/well (20,000 cells). See Figures 1 and 2 for other definitions.

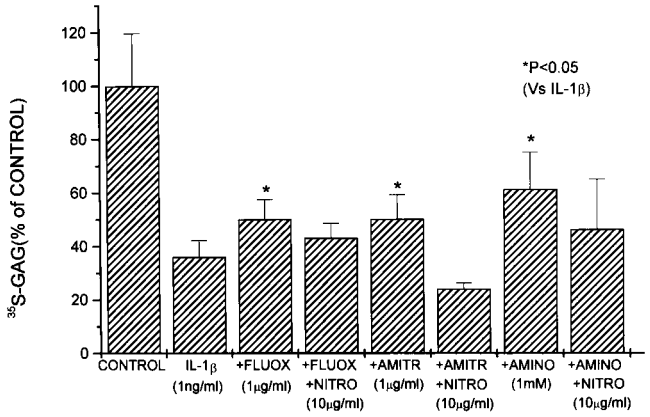

Figure 10. Effect of fluoxetine and amitriptyline (1 μg/ml) on sulfated glycosaminoglycan (^{35}S-GAG) synthesis by human cartilage in the presence of IL-1β (1 ng/ml), nitroprusside (NITRO) (10 μg/ml), and aminoguanidine (AMINO; 1 m*M*). Bars show the mean and SEM of 3 separate experiments (n = 3). The absolute mean ± SEM value in control cultures was 914 ± 179 disintegrations per minute (DPM)/mg cartilage and that in IL-1β–stimulated cultures was 331 ± 56.4 dpm/mg cartilage. See Figures 1 and 2 for other definitions.

PGE_2 production. Fluoxetine and amitriptyline (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) significantly and dose-dependently inhibited PGE_2 release in the media of human synovial cells in the presence of IL-1α plus TNFα (up to 88% inhibition) (Figures 3, 5, and 6). Indomethacin (5 μg/ml) completely abrogated PGE_2 production. Diazepam and nialamide (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) had no significant effect on stimulated PGE_2 production (Figure 7). Fluoxetine (3 μg/ml), amitriptyline (5 μg/ml), and hydrocortisone (5 μg/ml) significantly inhibited PGE_2 release in the media of human synovial tissue in the presence of LPS (3 μg/ml) (Figure 8).

HA production. Fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) and amitriptyline (1 μg/ml and 3 μg/ml) significantly and dose-dependently inhibited HA production in IL-1β (1 ng/ml) plus TNFα (30 ng/ml)–stimulated human synovial cells, as measured by ^{14}C-glycosamine uptake (Figure 9).

^{35}S-GAG synthesis. Both fluoxetine and amitriptyline (1 μg/ml) partially reversed IL-1β (1 ng/ml)–induced inhibition of ^{35}S-GAG synthesis by human cartilage cultures (Figure 10). The NO synthase inhibitor, aminoguanidine (1 m*M*), had a similar effect to that of fluoxetine and amitriptyline, while the NO donor, nitroprusside (10 μg/ml), reversed the effect of fluoxetine and amitriptyline (Figure 10).

At the above-mentioned concentrations, neither fluoxetine nor amitriptyline affected viability or had toxic effects when added alone to human synovial cell cultures, as measured by the XTT viability test (Table 1) and by trypan blue dye exclusion test (more than 95% of cells excluding trypan blue).

DISCUSSION

Although it has been known for a long time that some antidepressant drugs possess antiinflammatory (4) and analgesic (18,19) properties, their mechanism of

Table 1. Results of the XTT viability test*

	Mean ± SEM % of control
Control	100 ± 4.8
Fluoxetine 0.3 μg/ml	95.5 ± 5.0
Fluoxetine 1.0 μg/ml	127 ± 7.0
Fluoxetine 3.0 μg/ml	139 ± 5.0
Fluoxetine 10 μg/ml	144 ± 24.0
Amitriptyline 0.3 μg/ml	139 ± 15.5
Amitriptyline 1.0 μg/ml	106 ± 6.5
Amitriptyline 3.0 μg/ml	118 ± 15.0
Amitriptyline 10 μg/ml	117 ± 15.5

* The test measures the number of metabolically active cells by a colorimetric technique (17). Before applying the test, synovial cells were incubated for 72 hours at 37°C with fluoxetine or amitriptyline under the same conditions as in all other experiments described. The data represent the mean results of 4 separate experiments (6 wells for each drug concentration, 20,000 cells/well). The absolute mean ± SEM value in control cultures was 1.2 ± 0.05 optical density. It clearly appears that at all drug concentrations, the XTT viability test for synovial fibroblasts incubated with the 2 drugs showed more than 95% metabolically active cells.

action has remained unclear. An early study (4) attributed the antiinflammatory effect to their ability to inhibit catecholamine uptake. Later studies suggested an anti-PGE_2 effect for both tricyclic (e.g., clomipramine, amitriptyline) and serotonin reuptake inhibitor (e.g., fluoxetine) antidepressants (20) in experimentally induced inflammation in the rat. A similar antiinflammatory effect in the rat was described for carbamazepine (21), a drug sometimes used to combat depression. In patients with RA (22) and ankylosing spondylitis (23), amitriptyline was more effective than placebo in reducing articular pain and disease activity. However, another controlled, double-blind study showed no difference between placebo- and amitriptyline-treated RA patients regarding articular pain and tenderness (24).

Studies during the past few years clearly indicate an important role for NO in inflammation (25) and OA (26). NO mediates suppression of cartilage proteoglycan synthesis by IL-1 (27), and IL-1 stimulates its production by articular chondrocytes and synovial cells (28). Increased synthesis of HA by connective tissue cells is an integral part of connective tissue activation, as described by Sisson et al (29). There is a close correlation between stimulation of HA production and that of PGE_2, which are both induced by IL-1β and TNFα (30). We found elevated levels of HA in the sera of women with fibromyalgia (31), and others have reported elevated levels in patients with RA and OA, correlating with disease activity and disease progression (32,33). However, the pathophysiologic significance of this observation for fibromyalgia is still unclear. In the same vein, the significance of HA inhibition demonstrated by fluoxetine and amitriptyline in vitro (Figure 9) in fibromyalgia patients requires further clarification.

Furthermore, there are no data indicating increased cytokine levels in fibromyalgia patients. Addition of IL-1α plus TNFα to cultures of synovial cells derived from human synovial tissue induced stimulated release of NO in culture media (Figures 1 and 2). We used a combination of IL-1 and TNFα to augment the NO stimulation and to partially mimic a situation in OA and RA patients wherein both cytokines are presumably present. However, the cytokine concentrations used in our experiments were relatively high and were aimed at achieving measurable amounts of NO in our model.

Synergistic and additive effects of these 2 cytokines (Figure 9) were previously described (30). IL-1α and IL-1β showed very similar effects in our model (16). Fluoxetine (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) and amitriptyline (1 μg/ml and 3 μg/ml) significantly, and in a dose-dependent manner, inhibited stimulated NO release (Figures 1, 2, and 3). Nitric oxide synthase (NOS) inhibitors (aminoguanidine and ADMA) as well as hydrocortisone had an inhibitory effect on cytokine-stimulated NO, in the same manner as that of fluoxetine and amitriptyline (Figures 1 and 2), suggesting that fluoxetine, amitriptyline, and hydrocortisone inhibit NOS. Others, using a different model, have suggested that paroxetine, a serotonin reuptake inhibitor, is an NO inhibitor (34). It has been suggested that PGE_2 and NO do not mediate the synthesis of one another (35,36).

Fluoxetine and amitriptyline also inhibited stimulated PGE_2 release in the media of synovial cells (Figures 5 and 6) and synovial tissue (Figure 8), as did indomethacin (Figures 5 and 6) and hydrocortisone (Figure 8). In contrast, diazepam and nialamide (0.3 μg/ml, 1 μg/ml, and 3 μg/ml) had no effect on PGE_2 and NO release in the media of human synovial cells (Figures 4 and 7). It appears that this capacity of PGE_2 and NO inhibition is not shared by all psychotropic drugs, not even by all categories of antidepressants (nialamide, a monoamine oxidase inhibitor with antidepressant properties, had no effect in this respect). Both fluoxetine and amitriptyline (1 μg/ml) significantly ($P < 0.05$), but only partially, reversed inhibition of ^{35}S uptake induced in cartilage cultures by IL-1β (Figure 10). Since the NOS inhibitor, aminoguanidine, had an effect similar to that of fluoxetine and amitriptyline, and the NO donor, nitroprusside, reversed this effect, one may conclude that the effect of fluoxetine and amitriptyline is mediated by NO.

The results of our studies indicate that some antidepressant medications such as fluoxetine and amitriptyline, which represent 2 different classes of antidepressants, have the capacity of inhibiting cytokine-stimulated NO, PGE_2, and HA by human synovial cells and partially reversing cytokine-inhibited ^{35}S uptake by human articular cartilage. One of the questions to be answered in this respect is whether these medications are really present in the articular tissues and, if so, at what concentrations. To the best of our knowledge, no studies of their bioavailability in articular structures such as synovial fluid, synovial tissue, and articular cartilage are yet available. Measuring levels of fluoxetine and amitriptyline in the synovial fluid of patients receiving these medications is of interest. However, blood levels after administration of therapeutic doses of fluoxetine (37) and amitriptyline (38) have been determined, and these doses were very similar to the concentrations used in our experiments.

We suggest that the effects of these 2 drugs on inflammatory and noninflammatory rheumatic diseases

are related not only to PGE_2 inhibition, as previously reported, but also and perhaps even more so, to an inhibitory effect on NO production. Indeed, NO has been implicated in the creation of both central (39) and peripheral (40,41) pain. Furthermore, NO has been implicated as one of the important mediators of articular cartilage damage (27,28). Since amitriptyline and fluoxetine inhibit NO synthesis by connective tissue cells, such as both OA and RA synovial cells, we suggest that this may be a mechanism for their analgesic effects in OA, RA, and, perhaps, in fibromyalgia as well. Their apparent "chondroprotective" effect in vitro (Figure 10) requires further study in order to establish the in vivo value of these drugs.

In conclusion, our studies indicate that amitriptyline and fluoxetine inhibit production of NO, PGE_2, and HA by human synovial cells. Inhibition of NO and PGE_2 production is an important mechanism by which some antidepressant medications may affect pain, articular inflammation, and joint damage. Further studies are required in order to establish the bioavailability of these medications in human articular tissues and to establish the mechanism of their effects on NO, PGE_2, and HA production by stimulated connective tissue cells. The significance of their inhibitory effect on HA production in patients with fibromyalgia and other rheumatic diseases also warrants further elucidation.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Yoram Wolman and Mrs. T. Chernihovsky for their expert help in the measurements of nitric oxide.

REFERENCES

1. Carette S, McCain GA, Bell DA, Fam AG. Evaluation of amitriptyline in primary fibrositis: a double-blind, placebo-controlled study. Arthritis Rheum 1986;29:655–9.
2. Goldenberg DL, Mayskiy M, Mossey C, Ruthazer R, Schmid C. A randomized, double-blind crossover trial of fluoxetine and amitriptyline in the treatment of fibromyalgia. Arthritis Rheum 1996; 39:1852–9.
3. Viney LL, Westbrook MT. Coping with chronic illness. J Chronic Dis 1985;37:489–502.
4. Arrigoni Martelli E, Toth E, Segre AD, Corsico N. Mechanism of inhibition of experimental inflammation by antidepressant drugs. Eur J Pharmacol 1967;2:229–33.
5. Bianchi M, Panerai AE. Antidepressant drugs and experimental inflammation. Pharmacol Res 1996;33:235–8.
6. Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A, et al. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci U S A 1998;95:13313–8.
7. Ajuebor MN, Virag L, Flower RJ, Perretti M, Sjabo C. Role of inducible nitric oxide synthase in the regulation of neutrophil migration in zymosan-induced inflammation. Immunology 1998; 95:625–30.
8. Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, et al. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides. J Clin Invest 1995;96: 2357–63.
9. Castor CW, Bignell MC, Hossler PA, Roberts D. Connective tissue activation. XXI. Regulation of glycosaminoglycan metabolism by lymphocytes (CTAP-I) and platelet (CTAP-III) growth factors. In Vitro Cell Dev Biol 1981;17:777–85.
10. Van de Loo FAJ, Joasten LAB, van Lent PLEM, Arntz OJ, van den Berg WB. Role of interleukin-1, tumor necrosis factor α, and interleukin-6 in cartilage proteoglycan metabolism and destruction: effect of in situ blocking in murine antigen- and zymosan-induced arthritis. Arthritis Rheum 1995;38:164–72.
11. Feldmann M, Brennan FM, Maini R. Cytokines in autoimmune disorders. Int Rev Immunol 1998;17:217–28.
12. Jovanovic D, Pelletier JP, Alaaeddine N, Mineau F, Geng C, Ranger P, et al. Effect of IL-13 on cytokines, cytokine receptors and inhibitors on human osteoarthritis synovium and synovial fibroblasts. Osteoarthritis Cartilage 1998;6:40–9.
13. Nemeth ZH, Hasko G, Vizi ES. Pyrolidine dithiocarbamate augments IL-10, inhibits TNF alpha, MiP alpha, IL-12, and nitric oxide production and protects from lethal effect of endotoxin. Shock 1998;10:49–53.
14. Sugawara S, Shibazaki M, Takada H, Kosugi H, Eudo Y. Contrasting effects of an aminobisphosphonate, a potent inhibitor of bone resorption, on lipopolysaccharide-induced production of interleukin-1 and tumor necrosis factor alpha in mice. Br J Pharmacol 1998;125:735–40.
15. Ashab I, Peer G, Blum M, Wollman Y, Chernihovsky T, Hassner A, et al. Oral administration of L-arginine and captopril in rats prevents chronic renal failure by nitric oxide production. Kidney Int 1995;47:1515–21.
16. Yaron I, Meyer FA, Dayer J-M, Bleiberg I, Yaron M. Some recombinant human cytokines stimulate glycosaminoglycan synthesis in human synovial fibroblast cultures and inhibit it in human articular cartilage cultures. Arthritis Rheum 1989;32:173–80.
17. Roehm NW, Rodgers GH, Hatfield SM, Glasebrook AL. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J Immunol Methods 1991;142: 257–65.
18. Maciewicz R, Boukoms A, Martin JB. Drug therapy of neuropathic pain. Clin J Pain 1985;1:39–49.
19. Panerai AE, Bianchi M, Sacerdote P, Ripamanti C, Ventafridda V, De Conno F. Antidepressants in cancer pain. J Palliat Care 1991;7:42–4.
20. Bianchi M, Rossoni G, Sacerdote P, Panerai AE, Berti F. Effects of clomipramine and fluoxetine on subcutaneous carrageenin-induced inflammation in the rat. Inflamm Res 1995;44:466–9.
21. Bianchi M, Rossoni G, Sacerdote P, Panerai AE, Berti F. Carbamazepine exerts anti-inflammatory effects in the rat. Eur J Pharmacol 1995;294:71–4.
22. Frank RG, Kashani JH, Parker JC, Beck NC, Brownlee-Duffeck M, Elliott TR, et al. Antidepressant analgesia in rheumatoid arthritis. J Rheumatol 1988;15:1632–8.
23. Koh WH, Pande I, Samuels A, Jones SD, Calin A. Low dose amitriptyline in ankylosing spondylitis: a short term, double blind, placebo controlled study. J Rheumatol 1997;24:2158–61.
24. Grace EM, Bellamy N, Kassam Y, Buchanan WW. Controlled, double-blind, randomized trial of amitriptyline in relieving articular pain and tenderness in patients with rheumatoid arthritis. Curr Med Res Opin 1985;9:426–9.
25. Clancy RM, Amin AR, Abramson SB. The role of nitric oxide in inflammation and immunity. Arthritis Rheum 1998;41:1141–51.
26. Pelletier J-P, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, et al. Reduced progression of experimental osteoar-

thritis in vivo by selective inhibition of inducible nitric oxide synthase. Arthritis Rheum 1998;41:1275–86.
27. Taskiran D, Stefanovic-Racic M, Georgescu HI, Evans CH. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun 1994;200:142–8.
28. Stefanovic-Racic M, Stadler J, Evans CH. Nitric oxide and arthritis. Arthritis Rheum 1993;36:1036–44.
29. Sisson JC, Castor CW, Klavans JA. Connective tissue activation. XVIII. Stimulation of hyaluronic acid synthase activity. J Lab Clin Med 1980;96:189–97.
30. Meyer FA, Yaron I, Yaron M. Synergistic, additive, and antagonistic effects of interleukin-1β, tumor necrosis factor α, and γ-interferon on prostaglandin E, hyaluronic acid, and collagenase production by cultured synovial fibroblasts. Arthritis Rheum 1990; 33:1518–25.
31. Yaron I, Buskila D, Shirazi I, Neumann L, Elkayam O, Paran D, et al. Elevated levels of hyaluronic acid in the sera of women with fibromyalgia. J Rheumatol 1997;24:2221–4.
32. Emlen W, Niebur J, Flanders G, Rutledge J. Measurement of serum hyaluronic acid in patients with rheumatoid arthritis: correlation with disease activity. J Rheumatol 1996;23:974–8.
33. Sharif M, George E, Shepstone L, Knudson W, Thonar EJ-MA, Cushnaghan J, et al. Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38:760–7.
34. Finkel MS, Laghrissi TF, Pollock BG, Rong J. Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol Bull 1996; 32:653–8.
35. Jarvinen RA, Moilanen T, Jarvinen TL, Moilanen E. Endogenous nitric oxide and prostaglandin E_2 do not regulate the synthesis of each other in interleukin-1 beta stimulated rat articular cartilage. Inflammation 1996;20:683–92.
36. Hamilton LC, Warner TD. Interaction between inducible isoforms of nitric oxide synthase and cyclo-oxygenase in vivo: investigations using the selective inhibitors, 1400W and celecoxib. Br J Pharmacol 1998;125:335–40.
37. Burke WJ, Hendricks SE, McArthur-Cambell D, Jackues D, Stull T. Fluoxetine and norfluoxetine serum concentrations and clinical responses in weekly versus daily dosing. Psychopharmacol Bull 1996;32:27–32.
38. Rao ML, Deister A, Laux G, Staberock U, Höflich G, Moller HJ. Low serum levels of tricyclic antidepressants in amitriptyline and doxepin treated inpatients with depressive syndromes are associated with nonresponse. Pharmacopsychiatry 1996;29:97–102.
39. Ahn DK, Kim YS, Park JS. Central NO is involved in the antinociceptive action of intracisternal antidepressants in freely moving rats. Neurosci Lett 1998;243:105–8.
40. Anbar M, Gratt BM. Role of nitric oxide in the physiopathology of pain. J Pain Symptom Manage 1997;14:225–54.
41. Yoon YW, Sung B, Chung JM. Nitric oxide mediates behavioral signs of neuropathic pain in an experimental rat model. Neuroreport 1998;9:367–72.