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Role of Nociceptor Estrogen Receptor GPR30 in a Rat Model of Endometriosis Pain

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Abstract

Endometriosis, the most common cause of chronic pelvic pain, is an estrogen-dependent disease in which classic estrogen receptors (ERα, ERβ) play an important role. While recent evidence suggests that the novel G protein-coupled estrogen receptor (GPR30) also plays a key role in the progression of endometriosis, whether it is also involved in endometriosis pain is still unknown. Here we tested the hypothesis that GPR30 expressed by nociceptors contributes to endometriosis pain. Intramuscular injection of the GPR30 agonists raloxifene or 17β-estradiol produced a fast-onset, persistent, mechanical hyperalgesia at the site of the injection. Intrathecal antisense (AS) oligodeoxynucleotides (ODN), but not mismatch (MM) ODN, targeting mRNA for GPR30 markedly inhibited its protein expression in nociceptors and attenuated the mechanical hyperalgesia induced by local raloxifene or 17β-estradiol. Pre-treatment with the GPR30 antagonist G-36 also inhibited the hyperalgesia induced by raloxifene or 17β-estradiol, in naïve control rats. Surgical implant of autologous uterine tissue onto the gastrocnemius muscle, which induces endometriosis-like lesions, produced local mechanical hyperalgesia. Intrathecal AS, but not MM, ODN targeting GPR30 mRNA reversibly inhibited the mechanical hyperalgesia at the site of endometriotic lesions. Finally, intralesional injection of the GPR30 antagonist G-36 also inhibited the mechanical hyperalgesia at the site of ectopic uterine tissue. We conclude that local GPR30 agonists produce persistent mechanical hyperalgesia in naïve female rats, whereas local GPR30 antagonists inhibit mechanical hyperalgesia in a model of endometriosis pain. Thus, GPR30 expressed by nociceptors innervating ectopic uterine lesions might play a major role in endometriosis pain.

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Conflicts of interest statement

The authors have no conflict of interest with respect to this manuscript.

Keywords

17β-Estradiol; mechanical hyperalgesia; ectopic endometrium; raloxifene

1. Introduction

Endometriosis, a gynecologic disease which affects up to 10% of women of reproductive age, is a major cause of chronic pelvic pain [27]. Endometriosis pain is mainly manifest as mechanical hyperalgesia, including dysmenorrhea (painful menses), dyschezia (painful defecation), dysuria (painful urination) or dyspareunia (painful sexual intercourse) [21; 27]. While the pain in these patients is very disabling, causing huge health care related costs and lost work productivity [21; 27], available therapies are unsatisfactory. This is probably due, in part, to a complex pathophysiology [27; 54], which has made it difficult to get insight into underlying mechanism and therefore to develop safe and effective analgesic strategies.

Since endometriosis is mainly observed in women in their reproductive years and therapies that suppress ovarian function or inhibit estrogen synthesis produce relief of endometriosis symptoms [27; 54], it is widely regarded as an estrogen-dependent condition [10; 54]. However, the mechanisms underlying the contribution of estrogen to endometriosis pain remain to be elucidated [54] and data from clinical trials is somewhat contradictory. Indeed, while it is empirically established that therapies aimed to reduce estradiol can lessen endometriosis pain [54], unexpected worsening of pain has been observed in anti-estrogen therapy administered after excision of endometriosis lesions [55].

While many effects of estrogen are mediated by its action at its nuclear estrogen receptors (ER), alpha (ERα) and beta (ERβ), a growing body of evidence has demonstrated that many effects of estrogen are not due to ER-dependent transcriptional regulation (for a review see [49]). More recently, a novel estrogen receptor, the G-protein coupled receptor 30 (GPR30, also called G protein-coupled estrogen receptor 1, GPER1), has been identified [25; 49; 50]. Of note, GPR30 mRNA [35] and protein [20; 38; 39] are expressed by nociceptors and peripheral administration of selective GPR30 agonists or 17β-estradiol produces mechanical hyperalgesia within minutes after injection [32; 35]. Since endometriosis lesions are innervated by nociceptors [4; 8; 27; 54] and produce estrogen [17; 43; 44], we evaluated the role of GPR30 in endometriosis pain.

2. Material and Methods

2.1 Animals

Adult female Sprague Dawley rats (240–250 g at arrival; Charles River, Hollister, CA, USA) were used in these experiments. Housed in the Animal Care Facility at the University of California San Francisco, under environmentally controlled conditions (lights on 07:00–19:00 h; room temperature 21–23°C) they had food and water available *ad libitum*. Upon completion of experiments, rats were euthanized by inhalation of CO_2 followed by a bilateral thoracotomy. Animal care and use conformed to NIH guidelines (NIH Guide for the Care and Use of Laboratory Animals). The University of California San Francisco

Institutional Animal Care and Use Committee approved all experimental protocols. Concerted effort was made to minimize number and suffering of experimental animals.

2.2 Chemicals

Unless otherwise stated, all chemicals used in these experiments were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Surgical induction of endometriosis

The model of surgically-induced muscle endometriosis used here has been previously described in detail [4]. Briefly, anesthesia in female rats was induced with a mixture of ketamine hydrochloride (Putney, Portland, ME) and xylazine (AnaSed®, Lloid Laboratories, Shenandoah, IA) and maintained with isoflurane (Phoenix Pharmaceuticals, St. Joseph, M O). A local anesthetic block in the abdominal midline was performed by injecting 0.25% bupivacaine (Hospira, Lake Forest, IL) diluted in sterile isotonic saline (1:3). Under aseptic conditions a midline incision approximately 4 cm in length was performed. After laparotomy, the abdominal cavity was examined and the right uterine horn identified, exposed and isolated. After ligature of uterine blood vessels, a 1 cm segment was removed and immediately placed in a Petri dish containing 0.9% NaCl. The musculature of the abdominal wall was closed with single crossed stitches and the skin incision closed with horizontal mattress stitches. The excised uterine tissue was measured with a millimeter scale and opened longitudinally; a full thickness 3 × 3 mm square of uterine tissue was then removed and kept in saline. The implant was performed through the *biceps femoris* muscle allowing exposure of the underlying *gastrocnemius* muscle. The square of uterine tissue was sutured to the surface of the gastrocnemius muscle applying three to four single stitches using 5-0 nylon with the endometrial portion of the uterine tissue contacting the gastrocnemius muscle. After checking for hemostasis, the *b. femoris* muscle and the skin incision were sutured separately with single stitches.

2.4 Local injections

Rats were briefly anesthetized with 2.5 % isoflurane to facilitate the injection of drugs into the endometrial implant located on the gastrocnemius muscle (20 μl). The injection site was previously shaved and scrubbed with alcohol. The precise location of the uterine implant was identified by palpation and the tip of the needle directed to the base of the implant. Immediately after injection the skin puncture site was marked with a fine-tip indelible ink pen, so that the mechanical nociceptive threshold of the tissue underlying the injection site could be repeatedly tested. Solutions of 17β-estradiol (water soluble estrogen) were freshly prepared in 0.9% NaCl. Raloxifen (TSZCEMT, Framigham, MA) and G-36 (Azano Pharmaceuticals, Albuquerque, NM) were dissolved in 100% DMSO and subsequently diluted in 0.9% NaCl (final concentration of DMSO ≤5%), immediately before injection.

2.5 Antisense oligonucleotide (ODN) preparation and administration

The antisense (AS) ODN for the GPR30 gene, 5’-ATGTTCAGAGAGGTCCCCAG-3’ was directed against a unique region of the rat mRNA sequence. The corresponding NCBI Genbank accession number and ODN position within the cDNA sequence are NM_133573

and 182–201. The mismatch (MM) ODN sequence, 5'-A**G**GT**C**CAGA**A**AG**A**T**G**CC**A**AG-3' corresponds to the antisense sequence with 6 bases mismatched (denoted in bold). The AS and MM ODNs were purchased from Invitrogen (South San Francisco, CA). The ODNs were reconstituted in sterile saline (4 μg/μl) and stored at −20°C until use. Prior to injections, rats were anaesthetized with 2.5% isoflurane. A dose of 80 μg (injection volume 20 μl) of GPR30 AS or MM ODN was administered using a 0.3 ml syringe with a 29-gauge × ½" fixed hypodermic needle (Becton, Dickinson & Co., Franklin Lakes, NJ) inserted intrathecally, on the midline between the 4^{th} and 5^{th} lumbar vertebrae, once daily for 3 consecutive days. Intrathecal access was systematically confirmed by checking for a sudden tail flick [41]. Using this protocol we and others have previously demonstrated the knockdown of several different proteins in nociceptors, including the TTX-resistant sodium channel, $Na_V1.8$ [36], the MCP-1 receptor CCR2 [64], the mitochondrial fission regulator Drp-1 [23], and the polyadenylation element binding protein Cpeb [9].

2.6 Protein extraction and Western blotting

To confirm that the changes in the nociceptive responses associated with antisense oligonucleotide treatment for GPR30 mRNA are, indeed, due to a knockdown of the GPR30 expression in primary afferent nociceptors, a Western blot analysis was performed. L5 DRGs from rats treated with antisense or mismatch ODN over three consecutive days were excised 24 h after the last injection and transferred into homogenization buffer (150 mM NaCl, 10 mM EDTA, 2% SDS, 50 mM Tris-HCl pH 7.4) supplemented with a 2× protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The DRGs were homogenized with a hand-held plastic pistil in Eppendorf tubes, solubilized for 2 h under vigorous shaking in an Eppendorf mixer at room temperature (RT) and extracted by a 15 min centrifugation at 20,000 g in a tabletop centrifuge. Protein determination was performed using the micro BCA Protein Assay Kit (Pierce, Rockford, IL) with BSA as the standard. 30 μg of proteins/sample were denaturized by boiling in sample buffer (3% SDS, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.025% bromphenol blue, 62.5 mM Tris-HCl, pH 6.8) for 10 min and electrophoresed on 10% pre-cast polyacrylamide gels (Biorad) in 25 mM Tris containing 192 mM glycine and 0.1% SDS. Proteins were electrophoretically transferred to a nitrocellulose membrane by using the semi-dry method (transfer time 1 h at 10V, with 47.6 mM Tris, 38.9 mM glycine, 0.038% SDS and 20% (v/v) methanol). The blotting membranes were cut horizontally between 50 and 75 kDa, saturated by shaking in blocking buffer (5% BSA in Tris-buffered saline containing 0.1% Tween20 [TBST]) for 1 h at RT and probed with rabbit anti-PKCε (Santa Cruz Biotechnology, sc-214, 1:500), or rabbit anti-GPR30 (Abcam, ab 39742, 1:500) antibodies in blocking buffer at 4°C overnight. After washing with TBST (3 times at RT, 15 min each), both blots were probed with horseradish peroxidase conjugated anti-rabbit antibody 1:2500 (NA934V, GE Healthcare, Piscataway, NJ) in blocking buffer for 2 h at RT. The blots were washed with TBST (three times at RT, 15 min each) and the immunoreactivities were visualized using the enhanced chemiluminescence detection system (Pierce). Results were analyzed by computer-assisted densitometry and levels of GPR30 immunoreactivity were normalized with respect to the PKCE control levels in each sample.

2.7 Measurement of mechanical hyperalgesia

Mechanical nociceptive threshold in the implanted gastrocnemius muscle (or at the site of an intramuscular injection in naïve rats) was quantified using a digital force transducer (Chatillon DFI2; Amtek Inc., Largo, FL) with a custom-made 7 mm-diameter probe [4]. Rats were lightly restrained in a cylindrical acrylic holder with lateral slats that allow for easy access to the hind limb and application of the force transducer probe to the site of implantation in the belly of the gastrocnemius muscle. The nociceptive threshold was defined as the force (mN) required producing a flexion reflex in the hind leg. Baseline withdrawal threshold was defined as the mean of 3 readings taken at 5 min intervals and hyperalgesia values were calculated as a percentage of the baseline withdrawal threshold.

2.8 Statistical analysis

The analysis of the effect of intrathecal treatment was made by means of a two-way repeated measures analysis of variance (ANOVA), with one within subjects-factor (time) and one between-subjects factor (with two levels, treatment or control). If the ANOVA showed a significant interaction, Bonferroni's multiple comparisons test was performed to determine the basis of the differences. Graph Pad Prism 5.0 (GraphPad Software, Inc., San Diego, CA) was used to plot the graphics and to perform the statistical analysis. Data were plotted as mean ± S.E.M and statistical significance was set at $P < 0.05$.

3. Results

3.1 GPR30-mediated primary mechanical hyperalgesia

To evaluate whether estrogen produces a local proalgesic effect by acting on GPR30, we injected the GPR30 agonists 17β-estradiol or raloxifene [1; 45; 47] into the gastrocnemius muscle. One hour after injection of either 17β-estradiol (1–1000 ng, Fig. 1A) or raloxifene (0.1–1000 ng, Fig. 1B) a dose-dependent decrease in the mechanical nociceptive threshold was observed (n=6/group, $P < 0.001$, one-way ANOVA followed by Bonferroni's post-hoc test). The injection of the selective GPR30 antagonist G36 [19] dose-dependently (0.1–1 μg) prevented 17β-estradiol (100 ng/20 μl, n = 6, Fig. 1C) and raloxifene (100 ng/20 μl, n = 8, Fig. 1D) -induced mechanical hyperalgesia, indicating that the hyperalgesia induced by these compounds is GPR30-mediated.

3.2 Antisense knocks down GPR30 and blocks GPR30 agonist hyperalgesia

To assess whether the GPR30 agonists produce their proalgesic effect by action at the GPR30 receptor on nociceptors we injected intrathecally, either AS directed against GPR30 mRNA or its respective MM, daily for 3 consecutive days. Western blot analysis of DRG extracts from rats submitted to this treatment showed a marked decrease in the GPR30 immunoreactivity (−41±7%, $P = 0.0065$, Student's *t*-test, n = 6 rats/group, Fig. 2A), but no effect on housekeeping protein PKC epsilon (PKCE, Fig. 2A). In another group of rats, this ODN treatment did not affect the baseline mechanical nociceptive threshold (Post ODN, $P > 0.05$, Fig. 2B,C). However, the AS treatment compared to MM controls significantly attenuated the local hyperalgesia produced by both 17β-estradiol ($P < 0.001$, n = 6, Fig. 2B) and raloxifene ($P < 0.001$, n = 6, Fig. 2C), measured one hour after injection, indicating that

nociceptor expression of GPR30 is needed for GPR30 agonist-induced mechanical hyperalgesia.

3.3 Role of nociceptor GPR30 in endometriosis-like pain

Next, using the same antisense protocol, we evaluated whether GPR30 expressed by nociceptors is involved in the mechanical hyperalgesia exhibited by rats submitted to a model of endometriosis pain [3; 4; 33]. While groups of rats that were subsequently injected with AS or MM ODN (i.t.) exhibited similar preoperative and postoperative mechanical nociceptive thresholds, the group injected with AS exhibited a significant attenuation of the local hyperalgesia at the site of the endometriosis-like lesions ($P < 0.001$, n = 5, Fig. 3A). Such a significant anti-hyperalgesic effect was observed up to 5 days after the last AS injection ($P < 0.01$, n = 5, Fig. 3A).

Finally, to confirm the involvement of GPR30 in the mechanical hyperalgesia exhibited by rats submitted to a model of endometriosis pain, we explored the effect of the selective GPR30 antagonist G36. The intralesional injection of G36 (1 μg/20 μl), but not its vehicle, produced a marked increase in mechanical nociceptive threshold, which was significant at 15 min after injection (n = 6/group, $P < 0.001$, one-way ANOVA followed by Bonferroni's post-hoc test; Fig. 3B), and persisted for at least 24 h after injection (n = 6/group, $P < 0.001$, one-way ANOVA followed by Bonferroni's post-hoc test; Fig. 3B). Five days later, this anti-hyperalgesic effect was completely reversed (Fig. 3B).

4. Discussion

While endometriosis pain has long been considered to be estrogen-dependent [10; 27; 54], a safe and efficacious analgesic therapy based on this knowledge is still lacking. Indeed, clinical reports of therapies targeting estrogen are somewhat contradictory: on one hand, combined oral contraceptives containing estrogen can improve endometriosis pain [51; 54; 56] whereas raloxifene, which blocks some estrogen effects in a tissue-dependent manner, has been reported to markedly *shorten* the time to postoperative relapse for endometriosis pain [55]. Thus, the finding that local GPR30 agonists produce pain and that antagonism or disruption of GPR30 inhibits pain in a model of endometriosis provides a new explanation for those clinical observations.

4.1 GPR30 agonists produce mechanical hyperalgesia

Consistent with previous reports [32; 35], 17β-estradiol or raloxifene produced a GPR30-dependent primary mechanical hyperalgesia. This hyperalgesia was inhibited by pretreatment with a GPR30 antagonist or knockdown of nociceptor GPR30, indicating that nociceptors innervating the gastrocnemius muscle can be sensitized by GPR30 agonists to enhance their responsiveness to mechanical stimulation.

In good agreement with previous reports [20; 39], we observed the expression of GPR30 in DRG extracts, which was attenuated by intrathecal antisense treatment. The fact that primary hyperalgesia induced by local injection of 17β-estradiol or raloxifene was markedly attenuated by knockdown of GPR30, or the pretreatment with the GPR30 antagonist G36, indicates that this receptor is necessary and sufficient for the local proalgesic effect of

estrogen. This is in good agreement with findings from *in vitro* studies performed in cultured dorsal root ganglion and trigeminal neurons [22; 32; 38].

The estrogen receptor GPR30, which mediates the proalgesic effect of estrogen [22; 32; 35], has different tissue distribution and pharmacological profile compared to classic estrogen receptors (Dennis et al., 2009). For example, many GPR30 agonists act also as so-called selective estrogen receptor modulators or SERMs, meaning that these drugs can act like estrogen in some tissues and as an anti-estrogen in others [18]. Raloxifene, a clinically used SERM which also acts as a GPR30 agonist [1; 45; 47], exerts estrogen antagonist effects in breast tissue and is approved for the reduction of invasive breast cancer risk [5; 28]. It has also been successfully used to increase bone density in post-menopausal women [34], an osteoprotective effect mediated by GPR30 [45]. Interestingly, side effects of raloxifene include migraine/headache, generalized aches, mastalgia, pelvic pain, joint pain and leg cramps [28; 34; 37; 42], suggesting a pronociceptive effect for GPR30. This is in line with studies in rodents showing that GPR30 agonists produce nociceptive effects by acting directly on nociceptors [22; 32; 35; 38].

4.2 Endometriosis-like pain depends on nociceptor GPR30

The knockdown of GPR30 by antisense treatment in rats previously submitted to our model of endometriosis pain produced a marked attenuation of mechanical hyperalgesia. Additionally, intralesional administration of a GPR30 antagonist also inhibited mechanical hyperalgesia exhibited by these rats. This suggests that local activation of GPR30 contributes to mechanical hyperalgesia in endometriosis-like lesions. Since ectopic uterine tissue in humans [22; 32; 35; 38; 43; 44; 58] and rats [15] express the enzyme aromatase, which is critical for the synthesis of estrogen [10], locally produced estrogen acting on GPR30 might sensitize nociceptors innervating the endometriosis lesion. Indeed, while aromatase inhibitors and raloxifene are effective at reducing the size of endometriosis lesions [2; 12; 62], only aromatase inhibitors are successful to decrease pelvic pain symptoms in endometriosis patients [46].

It has been shown that estrogen not only can directly sensitize nociceptors but also allows other proalgesic mediators to display their sensitizing effect. For example, the expression of the leptin receptor in DRG neurons is estrogen-dependent [14] and injection of leptin produces persistent primary mechanical hyperalgesia [3]. Leptin is a proalgesic mediator present in peritoneal fluid of patients affected by endometriosis pain [6] and in endometriosis–like lesions in rats [3]. And, 17β-estradiol replacement recovers the hyperalgesic effect of local leptin attenuated by ovariectomy [3]. While evidence suggests that the expression of the leptin receptor is dependent on the activation of ERα [14], the contribution of GPR30 has not be ruled out. Indeed, some fast-onset effects of estrogen depend on calcium transient-induced activation of the RhoA/ROCK signaling pathway [13]. Furthermore, the GPR30 agonist G1 enhances the expression of transcripts encoding enzymes involved in the RhoA/ROCK signaling pathway [13; 24] and RhoA-dependent cytoskeletal remodeling (Chavalmane et al., 2010). Interestingly, the RhoA/ROCK signaling pathway has been recently described as an important route for the intracellular signaling of leptin [30]. And, concomitant increased levels of estrogen have also been reported in the

peritoneal fluid of patients affected by endometriosis pain [31]. Thus, leptin and estrogen could interact by means of the RhoA/ROCK signaling pathway through the GPR30 receptor. Such an interaction, concomitant to aberrant GPR30 signaling, has been proposed to explain adiposity, metabolic syndrome and prostate disease [60] but whether it also contributes to endometriosis pain remains to be determined.

4.3 Clinical implications

It has recently been reported that the expression of GPR30 is an important marker for the clinical progression of endometriosis [63]. This seems also be true for the involvement of this receptor in endometriosis pain, as revealed by clinical use of GPR30 agonists such as fulvestrant (Faslodex®, previously known as ICI 182,780) [57] and raloxifene [1; 45; 47]. While a phase II clinical study of the use of fulvestrant for endometriosis launched on 1999 never reported its outcome, another trial using raloxifene had to be stopped due to a significantly unfavorable outcome [29]. Indeed, based on the beneficial effect of raloxifene on bone density without endometrial stimulation, this drug or placebo were used as post-excisional therapy for women with endometriosis pain [55]. However, women treated with raloxifene had pelvic pain relapse significantly sooner than those taking placebo, without an association to biopsy-proven regrowth of the endometriosis lesions [55]. While unexpected, such an unfavorable outcome is consistent with previous basic reports indicating a pronociceptive role for GPR30 agonists [16; 22; 35; 38].

Additional mechanisms might have contributed to relapse of post-excisional endometriosis pain induced by raloxifene. For instance, histological analysis of endometriosis lesions in humans [40; 59] and in animal models [4] has revealed the expression of growth-associated protein-43 (GAP-43) in their innervating nociceptors. The GAP-43 expression contributes to neuronal sprouting, which is related to many forms of neuronal plasticity [7], including those underlying chronic pain [11; 26]. Increased levels of estrogen are known to enhance GAP-43 expression and axonal outgrowth in many brain regions [48; 52; 53]. It has been postulated that raloxifene binds to the raloxifene response element (RRE), a DNA sequence similar to the classic estrogen response element, and the gene encoding for GAP-43 contains an RRE-like sequence [61]. Thus, it is tempting to speculate that raloxifene treatment, in addition to its action on GPR30, could also stimulate nerve sprouting in nociceptors innervating excised endometriosis lesions, which might explain the earlier relapse in post excisional pain in raloxifene-treated patients (Stratton et al., 2008).

In summary, selective agonists of the GPR30 receptor produce mechanical hyperalgesia and inhibition or knockdown of the GPR30 receptor inhibits both ectopic uterine tissue-induced and estrogen-induced mechanical hyperalgesia. Estrogen acting through GPR30 might interact with leptin to produce enhanced nociceptive responses. Thus, strategies targeting GPR30 alone or in combination with anti-leptin therapy might be useful for the treatment of endometriosis pain.

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Summary

Evidence is provided to support the involvement of the G-protein coupled estrogen receptor 30 in nociceptor in endometriosis-induced primary mechanical hyperalgesia.

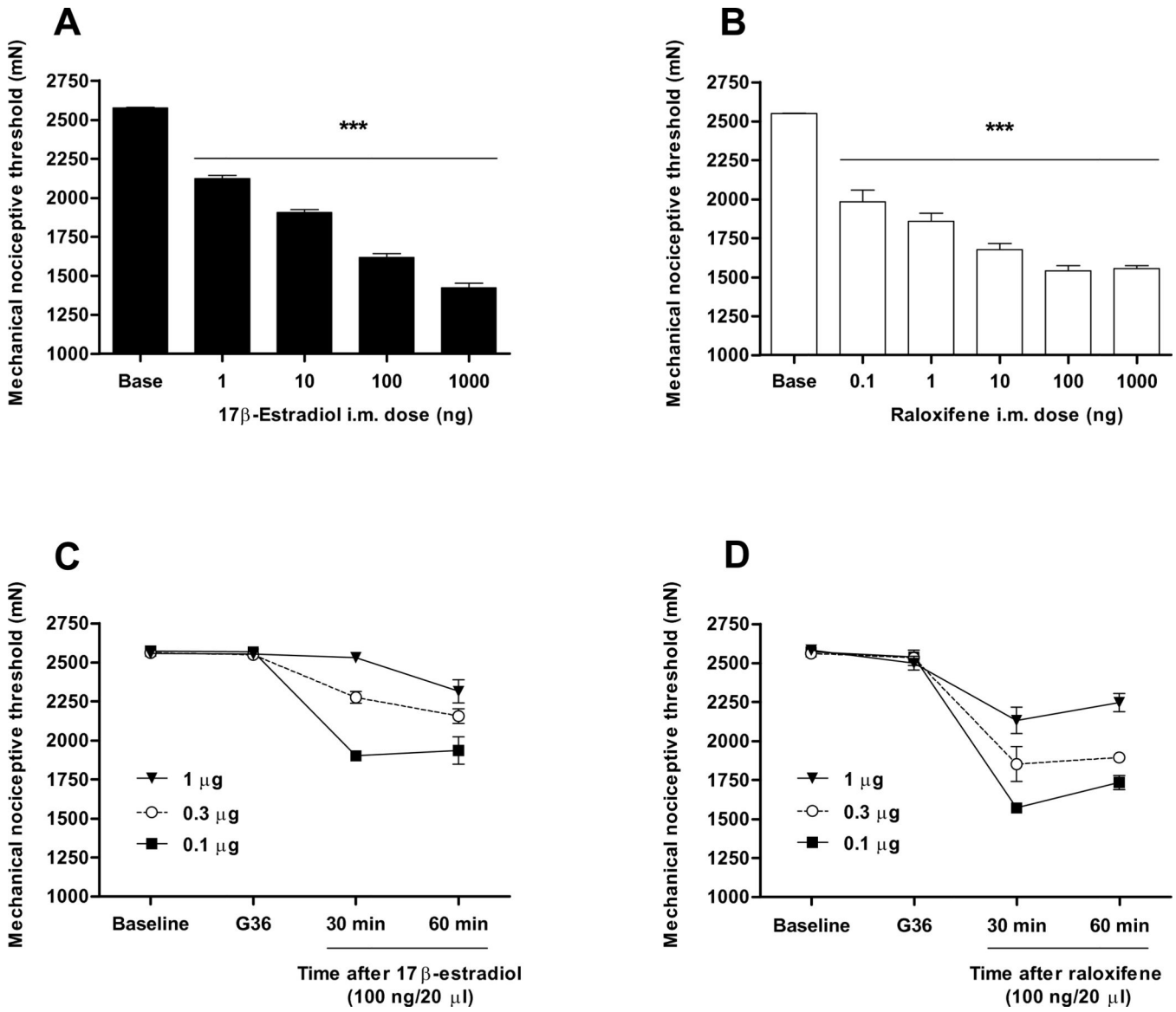

Figure 1. Intramuscular 17β-estradiol and raloxifene produce primary mechanical hyperalgesia by action at GPR30

Intramuscular injection of 17β-estradiol (**A**) or raloxifene (**B**) produces a dose-dependent mechanical hyperalgesia in intact female rats (n = 6 rats/group). Pre-treatment with the selective GPR30 antagonist G36, but not its vehicle (Veh), inhibits the hyperalgesia produced by both, 17β-estradiol (n = 6) (**C**) and raloxifene (n = 8) (**D**). ****P* < 0.001.

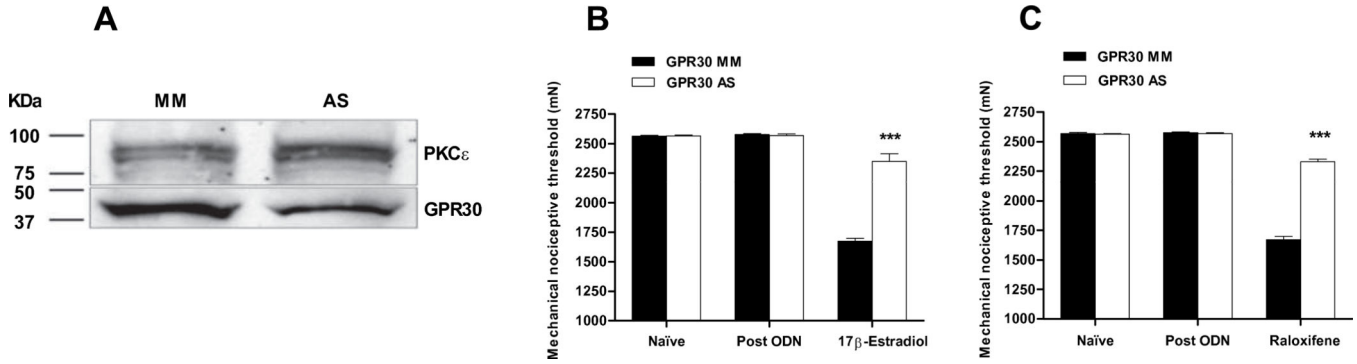

Figure 2. Effects of the intrathecal administration of antisense (AS) oligodeoxynucleotides (ODN) directed against GPR30 mRNA

(**A**) Western blot analysis of DRG extracts from rats submitted to intrathecal (i.t.) AS, but not mismatch (MM), ODN treatment (80 μg daily for 3 consecutive days) produced a marked a decrease in the GPR30 immunoreactivity ($P = 0.0065$, unpaired Student's *t*-test, n = 6 rats/group). Protein kinase C epsilon (PKCE) was used as housekeeping protein in this analysis. Using the same protocol, rats injected i.t. with AS (open bars) compared to the effect of MM (solid bars), directed against GPR30 mRNA (Post ODN) did not affect baseline nociceptive threshold but markedly attenuated the local hyperalgesia produced by intramuscular (**B**) 17β-estradiol (n = 6 rats/group), or (**C**) raloxifene (n = 6 rats/group). ***$P < 0.001$.

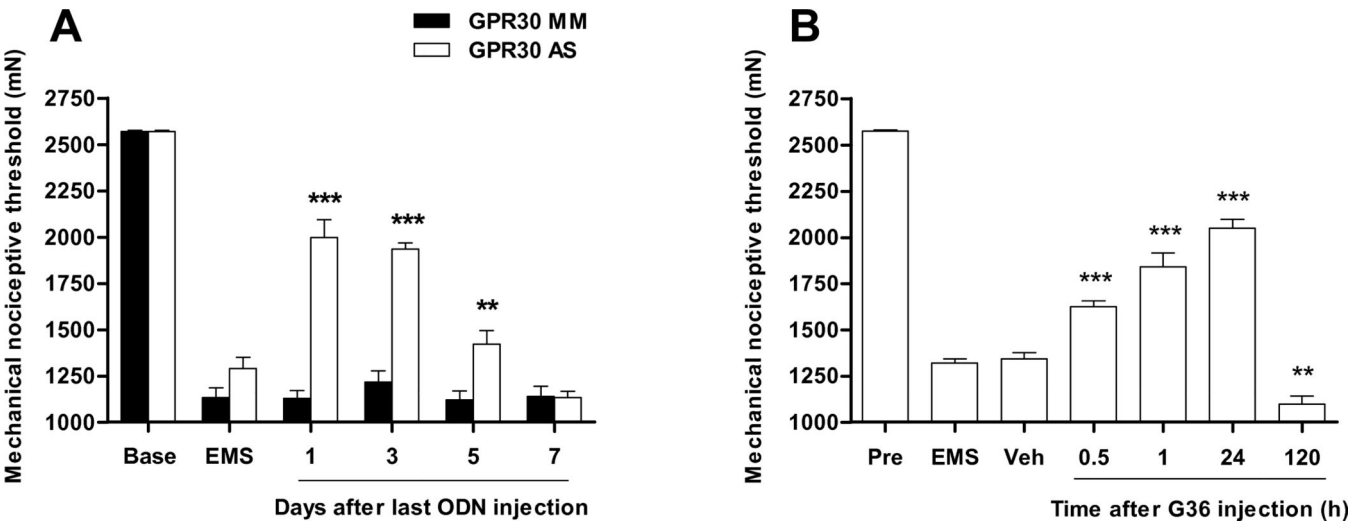

Figure 3. Effects of interventions targeting GPR30 on mechanical hyperalgesia induced by ectopic uterine tissue, a model of endometriosis pain

(**A**) While both groups of rats exhibited similar nociceptive thresholds before (base) and two weeks after the induction of experimental endometriosis (EMS), i.t. treatment (80 μg daily for 3 consecutive days) with AS (open bars), but not MM (solid bars), directed against GPR30 mRNA reversibly attenuated the endometriosis associated hyperalgesia. (**B**) In the same model, a single intralesional injection of the selective antagonist for GPR30 antagonist G36, but not vehicle (Veh), produced a persistent increase in the nociceptive mechanical threshold. $^{**}P < 0.01$; $^{***}P < 0.001$.