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Sex differences in inflammation and inflammatory pain in cyclooxygenase-deficient mice

Naomi L. Chillingworth,1 Scott G. Morham,2 and Lucy F. Donaldson1
1Department of Physiology, University of Bristol School of Medical Sciences, University Walk, Bristol, United Kingdom; and 2Myriad Genetics, Salt Lake City, Utah

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Chillingworth, Naomi L., Scott G. Morham, and Lucy F. Donaldson. Sex differences in inflammation and inflammatory pain in cyclooxygenase-deficient mice. Am J Physiol Regul Integr Comp Physiol 291: R327–R334, 2006.—There are two known cyclooxygenase (COX) genes, each encoding characterized enzymes, COX-1 and COX-2. Nonsteroidal anti-inflammatory drugs are commonly used as analgesics in inflammatory arthritis, and these often inhibit both cyclooxygenases. Recently, inhibitors of COX-2 have been used in the treatment of inflammatory arthritis, as this isoform is thought to be critical in inflammation and pain. The objective of this study was to determine the effect of COX-1 or COX-2 gene disruption on the development of chronic Freund’s adjuvant-induced arthritis and inflammatory pain in male and female mice. The effect of COX-1 or COX-2 gene disruption on inflammatory hyperalgesia, allodynia, inflammatory edema, and arthritic joint destruction was studied. COX-2 knockout mice (COX-2−/−) showed reduced edema and joint destruction in female, but not male, animals. In addition, neither male nor female COX-2−/− mice developed thermal hyperalgesia or mechanical allodynia, either ipsilateral or contralateral to the inflammation. COX-1 gene disruption also reduced inflammatory edema and joint destruction in female, but not male mice, although females of both COX−/− lines did show some bony destruction. There was no difference in ipsilateral allodynia between COX-1 knockout and wild-type animals, but female COX-1−/− mice showed reduced contralateral allodynia compared with male COX-1−/− or wild-type mice. These data show that the gene products of both COX genes contribute to pain and local inflammation in inflammatory arthritis. There are sex differences in some of these effects, and this suggests that the effects of COX inhibitors may be sex dependent.

alldynia; arthritis; hyperalgesia;

As COX-2 is induced at sites of inflammation and tumor growth, it is thought to be important in inflammatory and hyperplastic processes, whereas COX-1 is expressed widely in many tissues where it is involved in normal function, such as gastroprotection. Recent evidence, however, suggests that these “housekeeping” and “pathological” roles of COX-1 and COX-2, respectively, are oversimplified, as COX-2 may have roles in physiological functions and COX-1 may be involved in pathological processes (11, 40, 41, 77). In addition, elimination of COX-1 function both by pharmacological means and gene targeting does not result in gastrototoxicity, indicating a more complex interplay between COX-1 and COX-2 in gastric homeostasis than previously surmised (70, 78).

Chronic inflammatory arthritides, such as rheumatoid arthritis (RA), are characterized by dense inflammatory infiltrate in joints, with joint destruction and ankylosis. Pain in inflammatory arthritis is often treated with NSAIDs, or recently with COX-2-selective inhibitors (45) with the rationale that COX inhibition will reduce the inflammatory process. Although COX-2 has been found to be expressed in rheumatoid joints (36), the role of the individual COX isoforms and specifically COX-1 in this disease is less clear. Adjuvant-induced arthritis has been used for some time in the rat as a good model of RA, particularly with regard to joint destruction (16). We have developed this model in the mouse, to facilitate studies of RA in genetically modified animals (18).

During inflammation, pharmacological inhibition of cyclooxygenases has produced conflicting data on the role of these enzymes in nociception, with either COX-1 (34), COX-2 (72, 73), or both enzymes reported to be important (24, 26, 57, 58). COX-1, in addition to COX-2, may have important functions in inflammatory nociception, despite the current emphasis on COX-2 inhibitors as analgesics (59). All COX isoforms have been found in areas of the central and peripheral nervous systems appropriate to a role in nociceptive processing (9, 14, 15, 19, 31, 81). Pharmacological inhibition of central COX-2 results in reduced allodynia (69), although others have found inhibition of both COX isoforms decreases nociceptive responses (58).

Although we have not previously found a sex-dependent alteration of incidence of pathology in either rats or mice with Freund’s adjuvant-induced disease (18, 28), sex differences in the severity of disease have been reported in other models of

THERE ARE TWO KNOWN CYCLOOXYGENASE (COX) GENES, EACH ENCODING AT LEAST ONE FUNCTIONAL COX ENZYME. TRADITIONAL THINKING HAS HELD THAT COX-1 IS A CONSTITUTIVELY EXPRESSED ENZYME THAT PERFORMS HOUSEKEEPING FUNCTIONS, WHEREAS COX-2 IS AN INDUCIBLE ENZYME THAT HAS ROLES IN INFLAMMATION AND CELLULAR MITOSIS. THERE ARE KNOWN TO BE OTHER SPLENE VariantS OF THE COX GENES, BUT LITTLE IS CURRENTLY KNOWN ABOUT THE POSSIBLE FUNCTION OF THESE PROTEINS (68). COX ENZYMES ARE, TO VARYING DEGREES, THE MOLECULAR TARGETS OF THE NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS), WHICH ARE WIDELY USED IN THE TREATMENT OF INFLAMMATION AND PAIN (21, 36).

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MATERIALS AND METHODS

All procedures were carried out on adult mice (20–25 g) with disruption of either the COX-1 or COX-2 genes. The background strain of both COX-1–/– lines was the same, with homologous recombination carried out in 129ES cells, and founders from the C57/BL6 line. Both lines were backcrossed through at least 12 generations (55, 61). All experimental procedures, including housing of animals, conformed to United Kingdom Home Office legislation.

Genotyping of Knockout Mice

Mice were tail-tipped under local anesthesia, and DNA was extracted by incubation in proteinase K followed by phenol/chloroform extraction and ethanol precipitation. Primer sets for COX-1 wild-type and mutant alleles were as previously published (62). Wild-type 5′ primer (COX1–5′: AGGAGATGGCTGCTGAGTTGG), 3′ primer (COX1–3′: AATCTGACTTTCTGAGTTGCC), and mutant allele primer (COX1–Neo: GCAGCCTCTGTCATCTCTC) gave PCR products of 600 and 700 base pairs, respectively. Primer pairs for COX-2 wild-type and mutant alleles (COX-2 wild-type 5′: CATCCCTACCATTTGTAAGGAA; COX-2 wild-type 3′: TGTTTGAAGCTGCACTTCC); COX-2 mutant 5′: AGGATCTCCTGTCATCTCACCTTG; COX-2 mutant 3′: CCAAGCTCTTCGACAATACAGC) gave PCR products of 399 and 450 base pairs, respectively. In all studies, knockout mice were compared with wild-type age- and sex-matched control animals (n = 5–12 per group).

Chronic Inflammatory Arthritis in Mice

Chronic inflammatory arthritis was induced in mice using intradermal injection of Freund’s complete adjuvant (FCA; 250 μg attenuated Mycobacterium tuberculosis/100 μl mineral oil, made in-house) at two sites around the left tibiotarsal joint, under a brief halothane anesthesia (4% halothane in oxygen). Heat-killed Mycobacterium tuberculosis was a gift from Professor D. S. McQueen, University of Edinburgh and was originally obtained from the United Kingdom Ministry of Agriculture Fisheries and Food, now known as Department for Environment Food and Rural Affairs. This procedure results in unilateral tibiotarsal joint arthritis, with edema that is maintained for at least 20 days post-FCA injection in 100% of mice (18). Arthritic edema was assessed by direct measurement of tibiotarsal joint circumference using a tape measure every day and comparison was made to preinjection values. At the termination of the experiment, after 20 days, mice were killed by cervical dislocation. Hind paws were removed, fixed in formal saline, and then decalcified in Gooding and Stewart’s solution as previously described (18). Sections (10 μm) were stained with hematoxylin and eosin for histological analysis. Arthritis was assessed by scoring of histological sections of the tibiotarsal joints, according to features of arthritis present, as previously described (30) using a Nikon microscope. Histological sections were scored blind to treatment for the degree of inflammation as previously described (18) and according to the following scale: 0, no inflammation; 1, mild inflammatory infiltrate in skin and overlying tissues; 2, dense cutaneous inflammatory infiltrate, but no synovitis or arthritis; 3, synovitis; 4, hyperplastic synovium, inflammatory infiltrate/fibrin in the joint; 5, arthritis with destruction of articular tissues, pannus formation.

Thermal and Mechanical Inflammatory Nociception

Thermal and mechanical nociceptive tests were carried out before FCA injection and thereafter every 2 days on all mice. Thermal withdrawal latencies were determined using the Hargreaves apparatus, and mechanical nociceptive thresholds were determined using von Frey hairs, as previously described (18). Mechanical alldynia was assessed by determination of withdrawal thresholds to standard von Frey filaments, on both inflamed ipsilateral and noninflamed contralateral hindpaws. The plantar surface of each hindpaw was stimulated with von Frey filaments of increasing force until the filament that gave withdrawal responses to 3–5 stimuli was reached. This was then recorded as the force required to elicit a withdrawal response. Thermal hyperalgesia was assessed by the Hargreaves method (44). An infrared beam was placed under the plantar surface of each hindpaw, and the time taken for a withdrawal response to occur was recorded. Allodynia and hyperalgesia were assessed every 2 days. Comparison of nociceptive responses was made between wild-type and knockout mice and also to pre-FCA injection responses.

Data Analysis

All measures were compared with preinjection values using ANOVA followed by Dunnett’s post hoc test. Intergroup comparisons (within or between genotype or sex) were made using ANOVA followed by Bonferroni (intergroup comparisons) post hoc tests. All analysis was performed on raw data; in some cases, data are presented as percentage of control to prevent any lack of clarity due to slight differences in control values between genotypes and for easy comparison. In accordance with UK legislation, all animal numbers were the minimum necessary to achieve statistical significance. Acceptable statistical power for each measure was >0.85.

RESULTS AND DISCUSSION

Contribution of COX-1 and COX-2 Gene Products to the Development of Arthritis in Male and Female Mice

All wild-type mice developed arthritis to the same degree, regardless of sex. At 20 days after injection, all wild-type controls exhibited histological features of arthritis in the injected tibiotarsal joint, which were similar to features of RA, including hyperplastic synovium, dense inflammatory infiltrate into synovium, and evidence of bone remodeling (Fig. 1). Although female animals of both mutant genotypes showed histological evidence of inflammatory infiltrate and some evidence of bone destruction (Fig. 1, D and E), this was less apparent in male mice, which were essentially indistinguishable from wild-type controls, showing all the features found in wild-type animals. There was no histological evidence of contralateral inflammation in any animal, either wild-type or knockout. Thus, there was a significant sex difference in both COX-1 –/– and COX-2 –/– mice, in that in female mice, but not males, loss of either COX isoform significantly reduced the severity of the joint destruction seen after FCA injection. These findings and those on nociceptive behaviors in these animals are summarized in Table 1.
Cyclooxygenases and prostaglandins are intimately involved in the control of bone turnover, activating both osteoclasts and osteoblasts (43). The effect of disruption of either COX gene on the more severe bony changes seen in arthritic joints probably reflects the relationship between prostaglandin synthesis and bone turnover in both normal and inflammatory conditions (43).

These data were mirrored by the effect of COX gene disruption on joint edema. In female COX-1 and COX-2 −/− mice, joint swelling was significantly reduced compared with wild-type animals from day 6 (COX-2 −/−) and day 14 (COX-1 −/−) (Fig. 2A). Mean joint circumferences in female wild-type animals were 9.8 ± 0.4 mm at day 1, which was not significantly different from preinjection values in COX-1 −/− or COX-2 −/− animals. Wild-type joint circumferences increased to 17.9 ± 0.6 mm at 20 days, whereas female COX-1 −/− joint circumferences only increased to 14 ± 0.4 mm and female COX-2 −/− to 12.6 ± 0.9 mm (both at day 20). Male COX-1 −/− and COX-2 −/− mice showed no significant difference in the degree of edema from wild-type animals at all times (Table 1, Fig. 2B).

No study to date has examined sex differences and cyclooxygenases in arthritis; indeed, many studies use animals of only one sex and would therefore have not seen any sex-based differences (1, 82). In female mice, estrogen regulates PGI2 production by both COX-1 (65) and COX-2 (33), and this has been speculated to confer a protective effect in atherosclerosis (33). Pharmacological COX-2 inhibition (39, 62, 64, 82) or gene ablation prevents arthritis development; COX-2 gene disruption affects both bony destruction and edema in arthritis. The former effect is possibly attributable to the prevention of osteoclastogenesis by COX-2 inhibition (49).

There is conflicting evidence in the literature to implicate COX-2 in edema formation; in acute inflammation, COX-2 inhibition has been reported to reduce (39, 66) or have no effect upon edema (35, 38). Genetically modified mice in which COX-2 is upregulated show increased edema in the carrageenan model (42). The differences in edema occur earlier in COX-2 −/− compared with COX-1 −/− mice, and we hypothesize that the lack of inflammation-induced COX-2 induction could account for most of the differences in histological score and edema formation seen in these animals.

Table 1. Summary of the changes in inflammatory and nociceptive parameters measured in wild-type and COX knockout mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type</th>
<th>COX-1 −/−</th>
<th>COX-2 −/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Histological inflammation score</td>
<td>4.5±1.5</td>
<td>5±0.5</td>
<td>5±1</td>
</tr>
<tr>
<td>Ipsilateral joint edema</td>
<td>53% increase*</td>
<td>83% increase*</td>
<td>37% increase*</td>
</tr>
<tr>
<td>Ipsilateral mechanical withdrawal threshold</td>
<td>68% decrease*</td>
<td>50% decrease*</td>
<td>70% decrease*§</td>
</tr>
<tr>
<td>Contralateral mechanical withdrawal threshold</td>
<td>67% decrease*</td>
<td>60% decrease*</td>
<td>60% decrease*</td>
</tr>
<tr>
<td>Ipsilateral thermal withdrawal latency</td>
<td>65% decrease*</td>
<td>55% decrease*</td>
<td>20% decrease†§</td>
</tr>
</tbody>
</table>

Changes in oedema, mechanical withdrawal threshold and thermal withdrawal latency are all given as a mean % change at 17–20 days after induction of arthritis compared to the baseline measurement in the same group. Histological scores are given as median ± interquartile range. *Significant compared to preinjection baseline. †Significant compared to wild type. §Significant compared to the opposite gender, same genotype. §Significant compared to the same gender, other cyclooxygenase (COX) genotype. ND, not detectable.
**Cyclooxygenases and Sex Differences in Arthritis**

**COX-1 gene products have not been previously implicated in disease progression in arthritis; indeed, there is a paucity of evidence in the literature on potential functions of COX-1 in arthritis, although COX-1 inhibition does attenuate inflammation in other models (8, 55, 58). To our knowledge, this is the first report of both an important role for COX-1 gene products in the joint destruction associated with inflammatory arthritis and a sex difference in the function of this isoform.**

COX-1 is increased in RA and osteoarthritis in humans (50, 56), although not in all cases (22). The effect of COX-1 inhibition on edema is in agreement with other findings in animal models. In acute inflammation, edema is prevented by specific COX-1 inhibition (71). In more chronic inflammation, COX-1 expression is higher and more prolonged than COX-2 expression and is particularly important with their contribution to the regulation of inflammatory hyperemia, which will, of course, contribute to the level of edema (32), as increased blood flow to the joint will per se increase fluid filtration and possibly contribute to edema, according to Starling’s hypothesis. Recent work has reported that COX-1 expression is increased in adjuvant-induced arthritis in rats at 15 days postinjection (20), the same point at which COX-1 ablation has significant effects on arthritis and edema in our model, thus supporting our data on a contribution of COX-1 in the later stages of arthritis. Nonspecific pharmacological COX inhibition also affects more chronic increases in joint swelling (2, 12), suggesting, in agreement with our data, that no single COX isoform is responsible for this feature of the disease, but that both contribute.

**Contribution of COX Gene Products to the Development of Inflammatory Thermal Hyperalgesia and Mechanical Allodynia in Male and Female Mice**

**Thermal hyperalgesia.** All wild-type mice developed significant primary (at the site of inflammation) thermal hyperalgesia (Fig. 3A) from day 1 (reduction of withdrawal latency from 14 ± 1 s to 4 ± 0.5 s at day 6), and this was maintained throughout the period of arthritis. No animals developed contralateral thermal hyperalgesia: “mirror hyperalgesia” (data not shown).

**Fig. 2. Sex differences, cyclooxygenases, and inflammatory edema.** A: female COX-1 −/− (open squares) and COX-2 −/− (open circles) mice had significantly less joint edema than WT controls (solid squares), particularly in the chronic stages of the disease. Effects on edema appeared earlier in COX-2 −/− mice compared with COX-1 knockouts. (*P < 0.05 WT compared with COX-2 −/−; †P < 0.05 WT compared with both −/− phenotypes; ‡P < 0.05 WT cf COX-1 −/−). B: male COX-1 and COX-2 −/− animals showed no significant change in joint edema compared with WT controls.

**Fig. 3. Sex differences in thermal hyperalgesia.** Male COX-1 −/− mice have less hyperalgesia than females. A: COX-1 knockout mice (n = 12, open squares) developed thermal hyperalgesia that was significantly less than that seen in WT controls (n = 12, solid squares), and that resolved after day 15. COX-2 knockout mice did not develop significant primary thermal hyperalgesia at any point (n = 10, open circles) (*P < 0.05, **P < 0.01, ***P < 0.0001 ANOVA followed by Bonferroni’s post hoc test). B: female COX-1 −/− (n = 5, open diamonds) and WT (n = 5, solid squares) mice developed equivalent primary thermal hyperalgesia that did not resolve. Male COX-1 −/− mice (n = 7, open triangles) developed hyperalgesia that was significantly less profound than that in female COX-1 −/− or male (n = 7) wild-type mice (*P < 0.05, **P < 0.01 ANOVA followed by Bonferroni’s post hoc test).
Although both male and female COX-1 \(-/-\) mice developed significant ipsilateral thermal hyperalgesia, there was a significant difference in this measure between male and female COX-1 \(-/-\) mice (Fig. 3B). For example, reductions of withdrawal latency were from 14 ± 1 s at day 0 to 4 ± 0.5 s \(P < 0.001\) in females at day 8 but from 15 ± 1 s at day 0 to only 9 ± 1 s \(P < 0.001\) in males at the same time. Male mice exhibited significantly less ipsilateral hyperalgesia for most of the study (Table 1 and Fig. 3B). Thermal hyperalgesia in female animals was not significantly different from that seen in wild-type mice of either sex (Fig. 3B). Contralateral hyperalgesia was not observed in any animal, irrespective of genotype or sex (not shown).

COX-2 knockout mice did not develop ipsilateral thermal hyperalgesia at any point, and their response latencies were significantly less than wild types at all times (Fig. 3A).

**Mechanical allodynia.** All wild-type mice developed significant ipsilateral mechanical allodynia, as did COX-1 \(-/-\) mice (Fig. 4A). Von Frey thresholds dropped from 3 ± 0.5 g to 1 ± 0.1 g at day 1 \(P < 0.001\). Ipsilateral allodynia in wild-type and COX-1 \(-/-\) animals developed rapidly (24 h) and did not resolve over 20 days. There were no observed sex differences in ipsilateral allodynia, with male and female COX-1 \(-/-\) animals showing an equivalent degree of allodynia (Table 1 and Fig. 4C) \(P < 0.001\) ANOVA followed by Bonferroni’s post hoc test).

Neither male nor female COX-2 \(-/-\) mice developed ipsilateral allodynia at any point (Fig. 4A), suggesting that COX-2 has a significant role in the initiation and maintenance of allodynic responses in inflammatory arthritis.

Wild-type and male COX-1 \(-/-\) mice also developed significant contralateral mechanical allodynia in the uninflamed paw (Fig. 4B). Female mice had no significant reduction in threshold compared with control preinjection values, whereas male COX-1 \(-/-\) mice showed withdrawal thresholds of a similar magnitude to wild-type animals (Table 1, Fig. 4D).

COX-2 \(-/-\) animals did not develop secondary mechanical allodynia (Fig. 4B) irrespective of sex. Thus in the COX-2 \(-/-\) line, neither thermal hyperalgesia nor mechanical allodynia was seen in knockout mice. In the COX-1 \(-/-\) mice, females showed more profound changes in thermal hyperalgesia than males despite a greater degree of inflammation in inflammatory arthritis.

**Fig. 4.** Cyclooxygenases and mechanical allodynia. COX-1 ablation inhibits contralateral allodynia in male but not female mice. A: COX-1 knockout \((n = 12, \text{open squares})\) and WT \((n = 12, \text{solid squares})\) mice all developed significant primary mechanical allodynia, which was not seen in COX-2 knockout animals \((n = 10, \text{open circles})\) \(P < 0.05, \quad **P < 0.01, \quad +++P < 0.001\) ANOVA followed by Bonferroni’s post hoc test. B: COX-1 knockout \((n = 12, \text{open squares})\) and WT \((n = 12, \text{solid squares})\) mice develop significant secondary mechanical allodynia, which was not seen in COX-2 knockout animals \((n = 10, \text{open circles})\) \(P < 0.01, \quad +++P < 0.001\) ANOVA followed by Bonferroni. C: Male and female COX-1 knockout mice all developed significant primary alldynia equivalent to that of WT mice \((n = 7\) and 5, respectively, \(P < 0.001\) ANOVA followed by Dunnett’s test). There were no significant differences between groups. D: Male COX-1 knockout animals \((n = 7, \text{open triangles})\) developed contralateral mechanical allodynia equivalent to that seen in WT controls (solid triangles), but this was not seen in female COX-1 knockout animals \((n = 5, \text{open diamonds})\) \(P < 0.05, \quad **P < 0.01\) ANOVA followed by Bonferroni’s test.
males. In contrast, male and female COX-1 −/− mice developed equal levels of ipsilateral allodynia, whereas only male, and not females, showed significant contralateral allodynia (Table 1).

There is extensive literature on the role of cyclooxygenases in nociceptive processing, most of which supports an important role for the inducible isoform COX-2, in agreement with our data (25, 57, 58, 73, 83). The complete lack of hyperalgesia and allodynia in COX-2 mice is presumably a combination of the lack of both inducible COX-2 expressions at the site of inflammation and within the spinal cord; both areas in which COX-2 expression is increased during peripheral inflammation (9, 53), thus affecting both peripheral and central effects of prostaglandins on nociceptive neurons.

There is a paucity of evidence on a potential contribution of COX-1 to pain in arthritis, although COX-1 inhibition does attenuate nociception in some models (8, 58). Nonspecific pharmacological inhibition of COX has previously shown a greater contribution of prostaglandins to the development of allodynia as opposed to hyperalgesia (46).

Contralateral hyperalgesia and allodynia have been described in various models by different laboratories, and this “mirror pain” is considered to be a form of secondary hyperalgesia or allodynia (4, 6, 17, 52, 74). Secondary hyperalgesia and allodynia are mediated through activation of spinal neurons, and it is assumed that contralateral alterations in nociceptive processing are mediated through similar processes (29, 54). A large body of evidence, gathered over the past 7 years, on the actions of prostaglandins in the CNS [excellently reviewed by Vanegas and Schaible (75)] shows that centrally administered prostaglandins can result in allodynia. Intrathecal administration of both PGE2 and PGF2α results in allodynia. PGE2 is thought to exert this action through EP1 and EP3 receptors, as agonists at these receptors also cause allodynia (75). These effects require capsaicin-sensitive primary afferents to be functional. Recent data also support previous findings on the contribution of EP2 receptors to inflammatory secondary hyperalgesia and allodynia (67). Our data therefore suggest that the effect of COX-1 knockout on contralateral allodynia may be mediated through an effect on PGE2 production within the central nervous system, rather than a direct effect on the primary afferent, as COX-1 knockout in females had no effect on primary allodynia.

Analysis of our data demonstrates a sexually dimorphic role for COX-1 in joint destruction, edema, and nociceptive processing. There are many reports of sexual differences in pain responses, in humans and animals (10) and in both inflammatory and neuropathic pain (13, 51). For example, women are more likely to be prescribed NSAIDs than men in the treatment of osteoarthritis (27). Our data agree with studies that show that females show greater sensitivity to thermal (13, 23) and males greater sensitivity to mechanical stimulation (23). Sex differences in nociceptive processing have been attributable to estrogens and their suppression of nociceptive signaling in females (13, 23), together with some contribution from androgens (5). Sex differences in opioid analgesic efficacy have been previously reported (e.g., Ref. 37), as have sex differences in NSAID efficacy (76). It is, however, unclear from our data whether estrogens or androgens or both could mediate the effects we describe, as estrogens can regulate both COX isoforms to some degree. Testosterone can both enhance COX-2 expression (63) and inhibit prostaglandin production (60). We are not aware of any studies that have examined the role of testosterone in prostaglandin production in arthritis.

We have shown that COX gene disablement has effects on both disease severity and nociception, in a sex-specific manner. COX 1 knockout females have less severe bone destruction and inflammation. Males exhibit less thermal hyperalgesia than females, but they develop more contralateral mechanical allodynia. COX 2 knockout females have less severe bone destruction and inflammation and exhibit decreased thermal hyperalgesia compared with male knockouts or wild types of either sex.

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Present address of N. L. Chillingworth: Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY UK.

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