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# Involvement of peripheral cyclooxygenase-1 and cyclooxygenase-2 in inflammatory pain

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## Abstract

Pain-induced functional impairment in the rat (PIFIR) is a model of inflammatory and arthritic pain similar to that of clinical gout. Nociception is induced by the intra-articular injection of uric acid into the right hind limb, inducing its dysfunction. Animals then receive analgesic drugs and the recovery of functionality over time is assessed as an expression of antinociception. We have examined the role of peripheral prostaglandins synthesized by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in inflammatory pain using the PIFIR model. Rofecoxib (a selective COX-2 inhibitor) and SC-560 (a selective COX-1 inhibitor) both produced dose-dependent effects. When the inhibitors were administered before uric acid, they showed similar potency, but the antinociceptive efficacy of SC-560 was lower than rofecoxib; the best antinociceptive effects were obtained with the dose of 100 $\mu$g/articulation of each inhibitor (pre-treatment). In post-treatment (inhibitors administered after the uric acid), rofecoxib showed the least antinociceptive effect and SC-560 was more potent than rofecoxib. The inhibition of both COX-1 and COX-2 produced a more profound analgesic effect than the inhibition of either COX-1 or COX-2 alone. The present data support the idea that both COX isoforms contribute to the development and maintenance of local inflammatory nociception. Thus, it could be expected that inhibition of both COX-1 and COX-2 is required for non-steroidal anti-inflammatory drugs (NSAID)-induced antinociception in the rat. These findings suggest that the therapeutic effects of NSAIDs may involve, at least in part, inhibition of COX-1 and COX-2.

## Introduction

The enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) catalyse the conversion of arachidonic acid to prostaglandins (PGs), which play an important role in many biological systems, including homeostasis, integrity of the gastric mucosa, renal function and the inflammatory response (Hawkey 1999). Non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most widely prescribed drugs worldwide (Boynton et al 1988), non-selectively inhibit both COX-1 and COX-2 at standard anti-inflammatory doses (Yaksh et al 1998) and reduce the production of PGs, which sensitize nerve endings at the site of injury (Ferreira & Nakamura 1979). The analgesic effect of NSAIDs has traditionally been explained by an action at peripheral sites (Vane 1971) and, recently, by a central analgesic effect and an interaction with other systems involved in nociceptive processing (Björkman 1995; Pini et al 1997). COX-1 is constitutively expressed in most tissues including the kidney and the epithelial cells lining the gastrointestinal

tract. It is the only isoform of the enzyme expressed in platelets and it synthesizes PGs involved in the regulation of normal cell activity, including some physiological functions (Raskin 1999). COX-2 is absent from most normal tissues, but it is inducible by cytokines, growth factors and hormones (Fu et al 1990; Kujubu et al 1991), and it appears to produce PGs mainly at sites of inflammation (Seibert et al 1994). In experimental models where COX-2 is induced, an increase in PGs formation at sites of inflammation has been observed, whereas COX-1 is present constitutively and its expression is not changed. COX-2 expression has also been demonstrated in the synovial tissues from patients with rheumatoid arthritis.

Since the discovery of COX-2 in 1991, many COX-2 selective inhibitors have been developed and tested in clinical trials (Hawkey 1999). Selective COX-2 inhibitors, such as celecoxib and rofecoxib ([4-(4-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]), are promising agents for the treatment of arthritic pain and inflammation with potentially low risk of serious gastrointestinal side-effects (Chan et al 1999; Ryn & Pairet 1999). However, some data obtained in COX-1-deficient mice indicate that PGs synthesized by both COX-1 and COX-2 can contribute to the inflammatory response and that both isoforms have important roles in the maintenance of physiological homeostasis (Langenbach et al 1999; Wallace 1999). Thus, there is evidence indicating that the selective COX-1 inhibitor, SC-560 ([5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole]), did not affect acute inflammation or hyperalgesia produced by carrageenan in the rat footpad at doses that markedly inhibited COX-1 activity in-vivo, but it was able to reduce PGs at a level equivalent to that of the selective COX-2 inhibitor, celecoxib, which behaved as an effective analgesic in this model. This observation led to the suggestion that a centrally mediated mechanism involving COX-2 may be operative in inflammatory processes in addition to peripheral PGs (Smith et al 1998). Although the role of both isoforms has been well characterized in other models, little is known about their possible participation in inflammatory and arthritic pain similar to that observed clinically in gout.

The pain-induced functional impairment model in the rat (PIFIR) has been validated as an appropriate model to investigate the anti-inflammatory antinociceptive effects of NSAIDs and opioid drugs in arthritic pain (López-Muñoz et al 1993a). This pre-clinical assay provides a model of inflammatory and chronic pain similar to that found in clinical gout and it is useful to determine the efficacy, potency and duration of antinociceptive action of various drug classes. This experimental model has been employed in several studies of antinociception (Granados-Soto et al 1995; Hoyo-Vadillo et al 1995; López-Muñoz et al 1996, 1998; Aguirre-Bañuelos et al 1999), including the interaction of analgesic drugs (López-Muñoz et al 1993b, 1994; López-Muñoz 1994; Salazar et al 1995).

To investigate the role of COX-1 and COX-2 in arthritic pain, the present study was aimed at analysing the intra-articular effects of SC-560 and rofecoxib, which are selective inhibitors of COX-1 and COX-2, respectively, in the PIFIR model both before and after induction of the nociceptive process.

## Materials and Methods

### Animals

Female Wistar rats (Crl:(WI)BR), 180–200 g, were used in this study. They were housed in a temperature- and light-controlled room under a 12-h light–dark cycle (lights on at 0700 h) with free access to water and food. At 12 h before the experiments, food was withheld, but the rats had free access to drinking water. All experimental procedures followed the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Covino et al 1980) and the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann 1983), and were carried out according to a protocol approved by the local animal ethics committee. The number of experimental animals was kept to a minimum and they were used only once.

### Drugs

Uric acid was purchased from Sigma Chemical Co. (St Louis, MO). Rofecoxib and SC-560 were obtained from Laboratories Menarini S.A. (Barcelona, Spain). Uric acid was suspended in mineral oil, and rofecoxib and SC-560 were dissolved in 0.8% dimethylsulfoxide (DMSO).

### Measurement of antinociceptive activity

Antinociceptive activity was measured using the PIFIR model, which is described in detail elsewhere (López-Muñoz et al 1993a). Pain was induced by injection of 50 $\mu$L 30% uric acid into the knee joint of the right hind

limb (intra-articular administration) under light ether anaesthesia. An electrode was attached to the plantar surface of each hind paw between the plantar pads. Rats were allowed to recover from anaesthesia and they were forced to walk on a rotating stainless steel cylinder. The variable measured in this model was the time of contact between each of the rat's hind paws and the cylinder, which was rotated for 2-min periods. Recordings were made during this time and the rats were allowed to rest for 28 min between recording periods. Rats were forced to walk every 30 min over a 6-h period. Data are expressed as the percentage functionality index (FI%), that is the time of contact of the injected foot divided by the time of contact of the control left foot multiplied by 100.

### Experimental protocol

The experimental protocol consisted of three sets of experimental groups. In the first set of experiments, the dose–response curve for each drug was determined. Rofecoxib (25, 50 and 100 $\mu$g/articulation) and SC-560 (25, 50 and 100 $\mu$g/articulation) were administered into the knee joint of the right hind limb in a volume of 50 $\mu$L 20 min before the administration of uric acid in different groups of rats. For this purpose, rats were anaesthetized with ether. Each dose of the drugs was given to six animals, and adequate controls were performed with the vehicle (0.8% DMSO).

In the second set of experiments, the effects of the drugs before administration of uric acid (i.e. pre-treatments) were determined. One group of rats was administered with rofecoxib (100 $\mu$g/articulation) into the right paw (ipsilateral administration) and another group was administered rofecoxib into the left paw (contralateral administration) 20 min before the injection of uric acid. The same protocol was followed with SC-560 (100 $\mu$g/articulation) in two additional groups of rats. Moreover, the effect of the COX-1 selective inhibitor (ED33 = 32.14 $\mu$g/articulation) and the COX-2 selective inhibitor (ED33 = 36.29 $\mu$g/articulation) were obtained either individually or after co-administration in three additional groups of rats (i.e. pre-treatments). The functionality index after each treatment was recorded over a period of 6 h.

In the third set of experiments, the effects of the drugs were determined after the injection of uric acid (post-treatment). Rofecoxib (25, 50, 100 and 200 $\mu$g/articulation) and SC-560 (25, 50, 100 and 200 $\mu$g/articulation) were administered to different groups of rats 2.5 h after uric acid administration and the corresponding functionality index was recorded over a period of 4 h.

### Data presentation and statistical evaluation

FI% versus time curves were constructed for each treatment and the corresponding time course was determined. The area under the curve (AUC) for each treatment was calculated by the trapezoidal rule (Rowland & Tozer 1989). All values are the mean$\pm$ s.e.m. of six animals. The AUC for each treatment was compared using analysis of variance and by Dunnett's or Student's tests.

## Results

As previously observed in numerous studies using the PIFIR model (López-Muñoz et al 1993a, b, 1994, 1996, 1998; Granados-Soto et al 1995; Hoyo-Vadillo et al 1995), intra-articular administration of uric acid induced complete dysfunction of the right hind limb corresponding to a value of FI% = 0 in 2.5 h. This dysfunction was maintained throughout the entire experimental period, which comprised a further 3.5 h. In contrast, mineral oil (uric acid vehicle) did not produce any dysfunction in the rats. There were significant differences between the AUC obtained with uric acid and mineral oil treatments: 63.7$\pm$7.8 and 518.2$\pm$1.4 area units (au), respectively ($P < 0.01$, Student's test) (Figure 1). As expected, arthritic rats that received the SC-560 and rofecoxib vehicle (0.8% DMSO) 2.5 h after uric acid did not show any significant recovery of FI% during the observation period (data not shown).

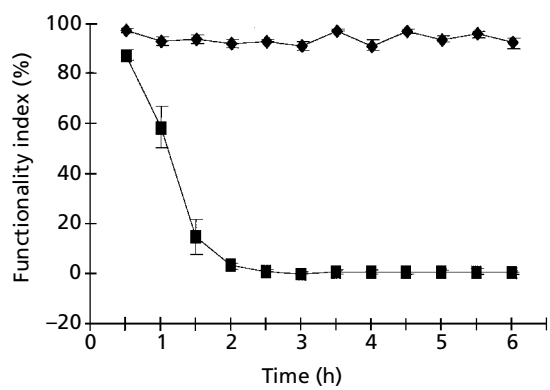


**Figure 1** Time course of controls in the pain-induced functional impairment model in the rat. Effects of 30% uric acid and mineral oil (uric acid vehicle) on the functionality index in rats. Chemicals were administered into the right hind knee. Uric acid (■) induced complete dysfunction 2.5 h after injection into the right hind limb (FI% = 0). Total dysfunction was maintained for a further 3.5 h. Mineral oil (◆) did not produce dysfunction. The observation time was 6 h. Data are expressed as the mean$\pm$s.e.m. of at least six experiments.

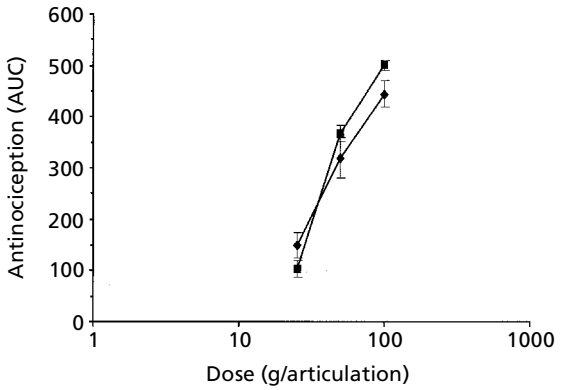


**Figure 2** Overall effect of SC-560 (selective COX-1 inhibitor; ◆) and rofecoxib (selective COX-2 inhibitor; ■) given 20 min before uric acid. Both drugs were administered into the right hind knee. In both cases, AUC increased in a dose-dependent manner. Both drugs showed similar potency, but the efficacy of SC-560 was lower than that of rofecoxib. The observation time was 6 h. Data are expressed as the mean±s.e.m. of at least six experiments.

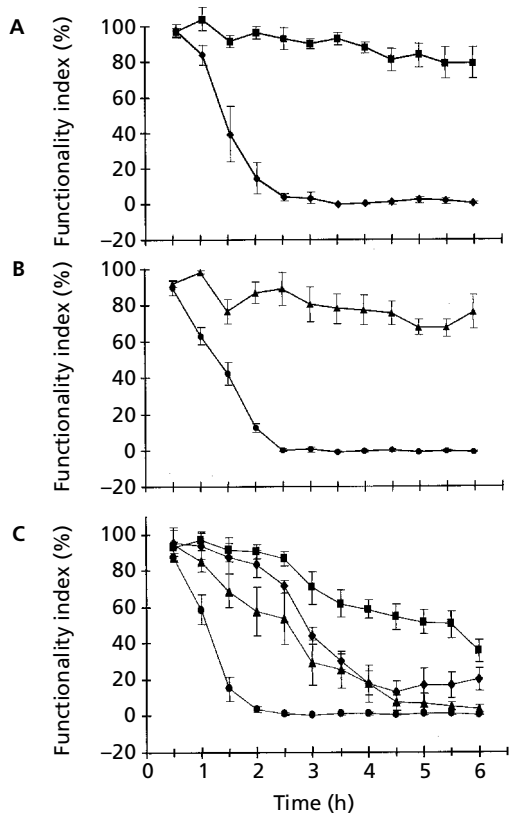


**Figure 3** Time course of the effect induced by rofecoxib (100 μg/articulation) (A) and SC-560 (100 μg/articulation) (B) administered into the paw both ipsilaterally (■, ▲) and contralaterally (◆, ●). Both drugs, when given ipsilaterally, caused a delay in the dysfunctional effect of uric acid. In contrast, contralateral administration of the drugs had no effect on uric acid-induced dysfunction. In addition, the effects of an ED33 dose of rofecoxib (▲) and SC-560 (◆) given alone and in combination (■) were obtained (C); ●, 0.8% DMSO (vehicle). Data are expressed as the mean±s.e.m. of six experiments. All drugs were administered 20 min before uric acid.

In the dose–response curves obtained after treatment with the COX inhibitors (pre-treatment : inhibitors administered 20 min before the injection of uric acid), it was found that, in both cases (COX-1 and COX-2 inhibitors), AUC increased in a dose-dependent manner. Both showed similar potencies, but the efficacy of SC-560 was less than that of rofecoxib: 445.2±25.5 and 500.5±10.0 au, respectively ($P < 0.05$, Student's test) (Figure 2). It was decided to analyse the time courses of 100 μg/articulation of rofecoxib and SC-560. Thus, pre-treatment with rofecoxib, administered 20 min before uric acid (ipsilateral), was able to keep the FI% at around 90 for 4.0 h. After this time, FI% diminished to around 80 for the last 1.5 h. Animals that received contralateral administration of rofecoxib developed progressive dysfunction of the injured limb. Dysfunction was total at 2.5 h after uric acid injection and it continued for a further 3.5 h. This treatment (left paw) was unable to prevent uric acid-induced dysfunction (Figure 3A). Interestingly, pre-treatment with SC-560, administered 20 min before uric acid (right paw), kept the FI% at around 100 during the first hour. Then, the FI% remained between 80 and 90 for 3.5 h, and between 70 and 80 during the last 1.5 h. The animals that received contralateral administration of SC-560 did not show any recovery from the impairing effect of uric acid. These rats developed progressive dysfunction of the injured limb, which was total at 2.5 h after the uric acid injection and remained the same for a further 3.5 h (Figure 3B). The results of the ipsilateral and contralateral drug administrations suggest that the effect of inhibition of PG synthesis on functionality takes place at a peripheral level only, because the contralateral administration was unable to prevent uric acid-induced dysfunction (i.e. they had a similar effect to that observed in control rats that received 30% uric acid). Analysis of the AUC values revealed that the treatments caused an effect that was significantly different compared with that observed after ipsilateral administration. These results are in keeping with the idea that, at the doses tested, the drugs had only a peripheral effect (Table 1). Pre-treatment with SC-560 (ED33), administered 20 min before uric acid (right paw), kept the FI% at around 80 for 2 h. Then, the rats developed progressive dysfunction of the injured limb. The animals that were pre-treated with rofecoxib (ED33), administered 20 min before uric acid

**Table 1** Effect of ipsilateral and contralateral administration of vehicle (0.8% DMSO), rofecoxib and SC-560 on the effect produced by a single administration of 30% uric acid.

| Drug | AUC (% h) | |
|---|---|---|
| | Ipsilateral administration | Contralateral administration |
| Uric acid (30%) | 63.67±7.78 | – |
| Vehicle (0.8% DMSO) | 75.14±10.30 | 67.73±12.11 |
| Rofecoxib (100 μg/articulation) | 500.51±9.95* | 77.14±10.14 |
| SC-560 (100 μg/articulation) | 445.19±25.46* | 97.49±9.51 |

Drug treatments were given 20 min before uric acid. Data are expressed as the area under the functionality index versus time curve (AUC) and are the mean±s.e.m. of at least six experiments. Significant differences with respect to controls were detected only after ipsilateral administration of rofecoxib and SC-560 (*$P < 0.01$, analysis of variance).

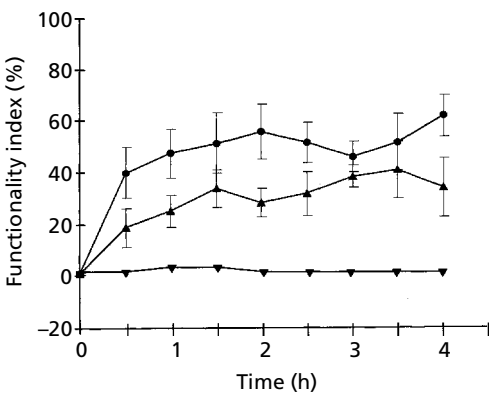


**Figure 4** Time course of the antinociceptive effect induced by vehicle and selective COX inhibitors measured as the recovery of the functionality index. Animals were treated with an intra-articular injection of vehicle (0.8% DMSO; ▼), or 100 μg/articulation of SC-560 (●) or rofecoxib (▲). Each treatment was injected into the ipsilateral paw 2.5 h after administration of 30% uric acid (FI% = 0). The vehicle did not produce any effect. SC-560 and rofecoxib produced antinociceptive effects for a period of 4 h. Under these conditions, SC-560 exhibited better antinociceptive effects than rofecoxib. Data are expressed as the mean±s.e.m. of at least six experiments.

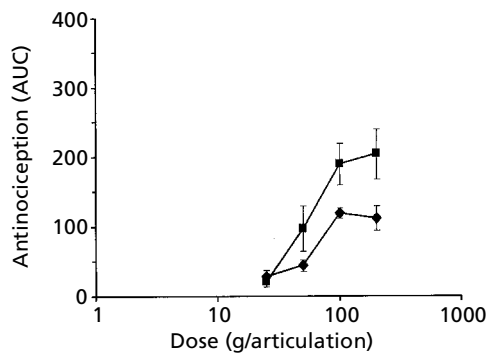


**Figure 5** Overall effect of SC-560 (selective COX-1 inhibitor; ■) and rofecoxib (selective COX-2 inhibitor; ◆) given 2.5 h after administration of 30% uric acid (FI% = 0). Both drugs were administered into the right hind knee. In the dose–response curves obtained after treatment with the COX inhibitors, it can be seen that in both cases, AUC increased in a dose-dependent manner. Rofecoxib and SC-560 showed different potencies (SC-560 was more potent than rofecoxib), and the efficacy of rofecoxib was lower than that of SC-560. The observation time was 4 h. Data are expressed as the mean±s.e.m. of at least six experiments.

(right paw), showed antinociceptive effects, but developed progressive dysfunction, which was total 4.5 h after the uric acid injection and remained the same for a further 1.5 h (Figure 3C). However, the co-administration of SC-560 (ED33) and rofecoxib (ED33) produced a greater analgesic effect than the inhibition of either COX-1 or COX-2 alone.

Both drugs were able to produce antinociceptive effects when given 2.5 h after uric acid, although SC-560 (100 μg/articulation) was more efficacious than rofecoxib (100 μg/articulation) (188.9±29.8 and 118.2±7.1 au, respectively; $P < 0.05$, Student's test). The FI% was maintained at around 50 and 30 after SC-560 and rofecoxib, respectively, for 4 h. In contrast, 0.8% DMSO (vehicle) was unable to produce antinociceptive effects under these conditions (0.8±0.1 au) (Figure 4). In the dose–response curves obtained after treatment with the COX inhibitors (post-treatment: inhibitors administered 2.5 h after the injection of uric acid), it was found that, in both cases (COX-1 and COX-2 inhibitors), AUC increased in a dose-dependent manner. Rofecoxib and SC-560 showed different potencies (SC-560 was more potent than rofecoxib), but the efficacy of

rofecoxib was lower than that of SC-560: 118.2±7.1 and 188.9±29.8 au, respectively ($P < 0.05$, Student's test) (Figure 5).

## Discussion

The purpose of the present study was to analyse the role of PGs synthesized by COX-1 and COX-2 in a model of inflammatory pain produced by intra-articular administration of uric acid into the rat knee joint (PIFIR). There is some evidence suggesting that PGs play an important role in the dysfunction induced by uric acid in this model, because some NSAIDs, including aspirin, acetaminophen, metamizol, ketorolac, flurbiprofen and ketoprofen (i.e. non-selective COX inhibitors) produce antinociception (López-Muñoz et al 1993b, 1998; López-Muñoz 1994). On the other hand, it is well known that COX-1 is constitutively expressed in most tissues where it plays a physiological role, whereas the inducible isoform, COX-2, is considered a pro-inflammatory enzyme and a major target for the treatment of inflammatory diseases (Masferrer et al 1994; Seibert et al 1994). However, selective inhibition of COX-2 only partially reduces the level of PGs at sites of acute or chronic inflammation in comparison with NSAIDs, which reduce PGs to undetectable levels (Anderson et al 1996). These results may suggest that COX-1 contributes significantly to the total pool of PGs at the site of inflammation and that there is minimal basal COX-1 activity at the site of injury.

Data obtained recently with COX-deficient mice indicate that both isoforms can contribute to the inflammatory response and that they have important roles in the maintenance of physiological homeostasis (Langenbach et al 1999). Evaluation of the anti-inflammatory effects of drugs selective for either COX-1 or COX-2 in carrageenan-induced pleurisy in rats showed that inhibition of COX-1 attenuated inflammation (Gilroy et al 1998). On the other hand, there is now evidence that COX-2 is expressed constitutively in many tissues and that it performs important physiological functions (Maslinska et al 1999). Thus, suppression of COX-2 with selective inhibitors could be expected to have some adverse consequences (Wallace 1999). The data obtained in our study confirmed that both isoforms (COX-1 and COX-2) could contribute to the development and maintenance of inflammatory pain. Selective inhibition of either COX-1 (with SC-560) or COX-2 (with rofecoxib) attenuated the nociception produced by uric acid in the rat. Indeed, in-vitro studies have shown that SC-560 has 1000-fold selectivity for COX-1 over COX-2 (Smith et al 1998). For this reason, SC-560 could be an important pharmacological tool with which to analyse the role of PGs synthesized by COX-1 in several experimental models. Our results suggest that PGs produced by COX-1 are as important as those synthesized by COX-2 in the development of inflammatory pain and maintenance of nociception in arthritic rats, because SC-560 was as efficacious as rofecoxib, a selective COX-2 inhibitor.

The nociceptive effect produced by both COX-1- and COX-2-derived PGs was peripheral, because contralateral administration of the drugs had no effect. A recent study compared the effects of SC-560 (a selective COX-1 inhibitor) and celecoxib (a selective COX-2 inhibitor) in the rat carrageenan footpad model (Fort 1999). Interestingly, therapeutic administration of SC-560 did not affect acute inflammation or hyperalgesia at doses that markedly inhibited COX-1 activity in-vivo. By contrast, celecoxib had anti-inflammatory and analgesic effects in that model. Paradoxically, both drugs reduced paw PGs to equivalent levels, but high levels of PGs were found in the cerebrospinal fluid after carrageenan injection, suggesting that, in addition to peripherally produced PGs, there may be a centrally mediated component to inflammatory pain that is mediated, at least in part, by COX-2 (Smith et al 1998). The differences between our results and those from other studies may be explained by the different routes of administration used. We administered the drugs locally into the knee joint, whereas other investigators administered them systemically. We intentionally followed this protocol to determine the role of peripheral PGs.

Data obtained in our laboratory with the PIFIR model suggest that rofecoxib does not appear to be more efficacious than conventional NSAIDs (unpublished observations). These observations are in agreement with those reported by Schuna & Megeff (2000). Despite this, COX-2 is now considered a pro-inflammatory enzyme, although evidence has also been presented suggesting that COX-2 may have anti-inflammatory properties under certain conditions (Gilroy et al 1999). In addition, using specific antibodies, Willoughby et al (2000) recently proposed the possible existence of a third isoform of COX (COX-3).

The present results using the PIFIR model are consistent with the idea that both COX isoforms (COX-1 and COX-2) can contribute to the nociceptive response. They also suggest that peripheral PGs derived from COX-1 and COX-2 play an important role in the development of inflammatory pain and maintenance of nociception produced by uric acid. On the basis of the above observations, it follows that the therapeutic

benefit of NSAIDs may be, at least in part, accounted for by the inhibition of both COX isoforms. It seems evident that selective inhibitors of COX-1 and COX-2 are not more efficacious than conventional NSAIDs.

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