Effects of Selective and Unselective Cyclooxygenase Inhibitors on Prostanoid Release from Various Rat Organs

IRMGARD TEGEDER, WERNER NEUPERT, HANS GÜHRING, and GERD GEISSLINGER

Center of Pharmacology, Johann Wolfgang Goethe-University of Frankfurt, Frankfurt am Main, Germany

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ABSTRACT

It has been assumed that cyclooxygenase-2 (COX-2) is solely responsible for inflammatory processes. Recently, this view has been challenged because COX-2-selective agents caused a delay of gastric ulcer healing and exacerbation of inflammation in rats. To further characterize organ-specific toxic effects of selective and nonselective COX inhibitors, we assessed the eicosanoid release from different rat organs ex vivo after oral administration of the COX-2-selective inhibitor NS-398 and the unselective COX inhibitors diclofenac, meloxicam, and ketorolac. Prostanoid and leukotriene release from tissue fragments of the stomach, kidney, lung, and brain were determined after ex vivo incubation of tissue fragments in Tyrode's solution for 10 min at 37°C. Ketorolac (0.1, 0.3, and 0.9 mg/kg) inhibited prostanoid release from all organs most potently and led to a significant increase of leukotriene release from the lung. Effects of diclofenac and meloxicam (1, 3, and 9 mg/kg each) were similar for all organs tested. At 9 mg/kg, 6keto-prostaglandin F (PGF)\textsubscript{1α} release from gastric mucosa was reduced by 79.1 ± 11.4 and 87.6 ± 7.7% and PGE\textsubscript{2} release from rat kidney was inhibited by 60.4 ± 6.8 and 78.6 ± 16.6% by diclofenac and meloxicam, respectively. NS-398 did not reduce prostanoid release from the lung. Consistent with the reported constitutive expression of COX-2, prostanoid release from kidney and brain was reduced by 20 to 30%. The release of 6keto-PGF\textsubscript{1α} from gastric mucosa was reduced by 34.7 ± 22.2% at 3 mg/kg and by 86.9 ± 12.7% at 9 mg/kg. At these doses, NS-398 has been previously shown to be COX-2-selective. Because PGF\textsubscript{1α} is the stable breakdown product of PGI\textsubscript{2}, these results suggest that COX-2 contributes to PGI\textsubscript{2} synthesis in the rat stomach.

The chronic use of classical nonsteroidal anti-inflammatory drugs (NSAIDs) for treatment of pain and inflammation is limited by their gastrointestinal and renal toxicity. Since the discovery of the “inducible” cyclooxygenase-2 (COX-2), great resources have been invested in developing selective inhibitors of this isoenzyme as gastrointestinal and kidney sparing anti-inflammatory and analgesic drugs. This is based on the premises that 1) COX-2 is solely responsible for prostanoid synthesis at sites of inflammation, and 2) COX-1 solely produces prostaglandins required for physiological organ functions such as cytoprotection of the gastric mucosa or regulation of renal blood flow. A number of recent studies, however, have raised questions about this “COX-2-selective” theory. A recent study comparing the anti-inflammatory activity of four different COX-2-selective compounds found that significant anti-inflammatory effects occurred only at doses that also inhibited COX-1. At these doses, gastric prostaglandin synthesis also was significantly suppressed and gastric mucosal erosions occurred (Wallace et al., 1998). In the rat formalin-induced nociceptive test, the selective COX-2 inhibitor NS-398 reduced the nociceptive response only at high doses (27 mg/kg) that most probably inhibit both isoenzymes (Euchenhofer et al., 1998). Mice lacking the gene for COX-2 exhibited inflammatory responses similar to those observed in wild-type mice (Morham et al., 1995), whereas mice devoid of the COX-1 gene showed diminished inflammatory responses (Langenbach et al., 1995). Thus, COX-1 may make important contributions to inflammatory responses.

In contrast, COX-2 appears to contribute to healing processes and physiological functions. During inflammation or ulceration in the gastrointestinal tract, COX-2 was shown to produce prostaglandins that are essential for repair (Mizuno et al., 1997; Schmassmann et al., 1998). In these circumstances, inhibition of COX-2 caused a delay of ulcer healing and exacerbation of inflammation (Mizuno et al., 1997; Schmassmann et al., 1998). Importantly, the inhibition of ulcer healing was observed at doses of the selective COX-2 inhibitor L745,337 previously shown to be COX-2-selective (Chan et al., 1995). Prostaglandins produced by the colonic mucosa in a rat model of colitis were largely derived from

ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; COX-2, cyclooxygenase-2; PGF, prostaglandin F; LT, leukotriene; TX, thromboxane.
COX-2. Daily treatment with selective COX-2 inhibitors resulted in significant inhibition of prostaglandin synthesis and exacerbation of colitis (Reuter et al., 1996). In carrageenan-induced pleurisy in rats indomethacin and NS-398 inhibited inflammation at 2 h but caused significant exacerbation at 48 h (Gilroy et al., 1999). In clinical studies, however, COX-2-selective agents had potent anti-inflammatory effects and caused significantly fewer gastrointestinal ulcers than nonselective drugs (Simon et al., 1998).

For the kidney, it was generally accepted that physiological production of prostaglandins is mediated by COX-1. However, in the absence of inflammation, low levels of COX-2 mRNA also were detectable (Yang et al., 1999). When animals were chronically sodium restricted, COX-2 expression increased, predominantly in the region of the macula densa (Harris et al., 1994). Dehydration stimulated COX-2 expression in renal inner medulla (Yang et al., 1999). In salt-depleted subjects, the selective COX-2 inhibitor celecoxib caused sodium and potassium retention and short-term transient decreases in renal blood flow and glomerular filtration rate (Rossat et al., 1999). The intrarenal distribution of COX-2 and its increased expression in response to sodium or water restriction suggest that in addition to its proposed role in inflammatory responses, COX-2 may play an important role in the regulation of salt and volume homeostasis.

To further characterize the organ specific toxicity of different NSAIDs, we have investigated the dose-dependent release of various eicosanoids that are typical for the rat stomach, kidney, lung, and brain after administration of the COX-2-selective inhibitor NS-398 and the nonselective COX inhibitors meloxicam, diclofenac, and ketorolac.

Materials and Methods

Drugs

Diclofenac sodium was obtained from Sigma Chemical Co. (Sigma-Aldrich, Steinheim, Germany). Meloxicam was extracted from commercially available tablets. Purity was determined by HPLC, and identity by mass spectrometry. Ketaoral was obtained from Syntex Chemicals (Boulder, CO) and NS-398 from Cayman Chemicals (Ann Arbor, MI).

Rat Organ Experiments Ex Vivo

Drug Administration. Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 180 to 230 g were deprived of food for 24 h with free access to tap water. Drugs were moistened with soybean lecithin and dissolved in a 1:1 mixture of 2% Tylose and phosphate buffer (pH 7.4). Doses of 1, 3, and 9 mg/kg diclofenac, meloxicam, and NS-398 and 0.1, 0.3, and 0.9 mg/kg ketorolac (n = 4–8 in each group) were administered orally in a volume of 500 µl. Doses of 1, 3, and 9 mg/kg diclofenac, with soybean lecithin and dissolved in a 1:1 mixture of 2% Tylose and food for 24 h with free access to tap water. Drugs were moistened

Tissue Preparation. Tissue preparation was done as described previously (Pallapies et al., 1995). In brief, after the animals were deeply anesthetized most of the blood was withdrawn by cardiac puncture. Brain without cerebellum, stomach, lungs, and kidneys were then rapidly excised. The organs were washed briefly in ice-cold saline and then cut into tissue fragments (1–3 mm³). The tissue fragments were weighed and then incubated in oxygenated Tyrode’s solution at 37°C for 10 min. In detail, ~50 mg of gastric mucosa was incubated in 0.5 ml, ~130 mg of lung or kidney fragments in 1.0 ml, and fragments of one-half of the brain (500–700 mg) were incubated in 3.0 ml of the oxygenated Tyrode’s solution. Incubation was stopped by transferring the incubation medium to a separate tube that was kept at ~20°C until analysis of the prostanooid and leukotriene concentrations. For each rat, two tissue fragments of each organ (right and left side for brain, lung and kidney, two distinct locations of the stomach) were prepared. In all experiments, the ethics guidelines for investigations in conscious animals were obeyed and the procedures were approved by the local ethics committee.

Enzyme Immunoassay. Release of prostanooids and leukotrienes from the tissue fragments into the incubation medium was determined by commercially available enzyme immunoassay kits (Cayman Chemicals). The release of thromboxane (TX) B₂, the stable degradation product of TXA₂, into the incubation medium was determined for brain and lung. Release of 6-keto-prostaglandin F₁α (PGF₁α), the stable breakdown product of PGI₂, was determined for lung, kidney, and gastric mucosa. PG₂E and PG₂Fα release was determined for kidney and brain, respectively. Thus, for all organs prostanooids were selected for quantitative determination that have been shown to comprise a major fraction of the total prostanooids released under the experimental conditions used (Anhut et al., 1978; Ferreri et al., 1983; Dembinska-Kiec et al., 1984). In addition to the prostaglandins, release of the leukotrienes (LT) LTB₄ and LTC₄ into the incubation medium of the lung was determined. All results were calculated as picograms per milligram wet weight per 10 min.

The reliable limits of quantification for TXB₂, PG₂E, 6-keto-PGF₁α, and PG₂Fα were 10, 20, 15, and 14 pg/ml, respectively. The mean percentage deviation over the calibration range of 10 to 1000 pg/ml was 11, 14, 12, and 15%, respectively. The limits of quantification for LTB₄ and LTC₄ were 12 and 10 pg/ml, respectively, and the mean percentage deviation over the calibration range of 10 to 750 pg/ml was <15%.

Immunohistochemistry

Rats were euthanized with CO₂ and perfused intracardially with 0.1 M PBS followed by 4% paraformaldehyde in 0.1M phosphate buffer. The stomach was removed, postfixed for 4 h, and cryoprotected in 15% sucrose overnight at 4°C. Sections (10 µm) were cut on a cryostat, thawed onto poly(l-lysine)-coated slides, and air dried. Cryostat sections were incubated in PBS containing 1% BSA, 0.5% Triton X-100, and 10% donkey normal serum for 1 h at room temperature. Between the following steps, slices were washed extensively in PBS. Sections were incubated overnight with primary antibodies directed against rat COX-2 (1:500, M-19; Santa Cruz, Heidelberg, Germany). Binding sites were visualized with Cy3-labeled donkey anti-goat IgG (1:200; Dianova, Hamburg, Germany). Slices were embedded in glycerol/PBS, coverslipped, and assessed by confocal laser scanning microscopy (BioRad MRC 1000 attached to a Nikon Diaphot 300) equipped with a krypton-argon laser (Ion Laser Technology, Salt Lake City, UT). Sections were imaged with a filter appropriate for the specific visualization of Cy3 (568-nm excitation, filter 605 DF 32).

Statistics

For statistical evaluation, SPSS version 8.01 for Windows (SPSS Inc., Chicago, IL) was used. Mean values of the right and left organ fragments or from the two stomach samples, respectively, were used for analysis. The data were submitted to univariate ANOVA followed
Results

The percentage inhibition of eicosanoid release from stomach, kidney, lung, and brain is depicted in Figs. 1-5. A statistically significant inhibition of eicosanoid release compared with vehicle is marked with an asterisk (*P < .05, **P < .01, ***P < .001).

**Gastric Mucosa.** After administration of vehicle, 439.5 ± 260.7 pg 6-keto-PGF$_{1\alpha}$/mg wet weight/10 min was released from fragments of rat gastric mucosa. Figure 1 shows the dose-dependent percentage inhibition of 6-keto-PGF$_{1\alpha}$ release from the stomach for ketorolac, diclofenac, meloxicam, and NS-398. As expected from previous studies, ketorolac inhibited 6-keto-PGF$_{1\alpha}$ release more potently than the other three drugs, reaching 94.1 ± 5.1% inhibition at 0.9 mg/kg. Inhibition of 6-keto-PGF$_{1\alpha}$ release by diclofenac and meloxicam was very similar, with a maximum inhibition of 79.1 ± 11.4 and 87.6 ± 7.7%, respectively, at the highest dose tested (9 mg/kg). NS-398 did not inhibit 6-keto-PGF$_{1\alpha}$ release at 1 mg/kg. However, at 3 and 9 mg/kg it significantly inhibited prostanooid release (34.7 ± 22.2 and 86.9 ± 12.7% inhibition, respectively) and reached a maximum inhibition similar to that achieved with meloxicam and diclofenac. Statistically, all of the four drugs significantly inhibited 6-keto-PGF$_{1\alpha}$ release compared with vehicle. In detail, ketorolac inhibited 6-keto-PGF$_{1\alpha}$ release at 0.1 (P < .001, confidence interval for differences 47.0–133.4%), 0.3 (P < .001, confidence interval for differences 63.7–122.9%), and 0.9 mg/kg (P < .001, confidence interval for differences 64.5–123.7%). For diclofenac and meloxicam, only the two higher doses of 3 and 9 mg/kg significantly reduced 6-keto-PGF$_{1\alpha}$ release (1 mg/kg diclofenac, P = 1, confidence interval for differences −21.1–65.3%; 3 mg/kg, P < .001, confidence interval for differences 43.2–98.6%; and 9 mg/kg, P < .001, confidence interval for differences 51.4–106.8% and 1 mg/kg meloxicam, P = 0.38, confidence interval for differences −8.2–78.1%; 3 mg/kg, P < .001, confidence interval for differences 28.9–88.2%; and 9 mg/kg, P < .001, confidence interval for differences 59.9–115.3%). For NS-398, 6-keto-PGF$_{1\alpha}$ release after 1 mg/kg did not significantly differ from vehicle (1 mg/kg, P = 1, confidence interval for differences −21.6–64.8%). However, 3 and 9 mg/kg produced a significant reduction of prostanooid release (3 mg/kg, P = .029, confidence interval for differences 1.7–72.3%; 9 mg/kg, P < .001, confidence interval for differences 59.2–114.8%).

**Kidney.** After administration of vehicle, fragments of rat kidney released 379.5 ± 189.0 pg PGE$_2$/mg wet weight/10 min and 141.3 ± 61.2 pg 6-keto-PGF$_{1\alpha}$/mg wet weight/10 min. The percentage inhibition of PGE$_2$ and 6-keto-PGF$_{1\alpha}$ release from kidney is shown in Fig. 2, A and B, respectively. The results for both prostanooids are very similar. Again, ketorolac is the most potent of the four NSAIDs tested, leading to a dose-dependent 95.3 ± 7.2, 97.3 ± 3.0, and 98.9 ± 0.9% inhibition of PGE$_2$ and a 84.7 ± 5.0, 95.7 ± 4.1, and 97.6 ± 2.1 inhibition of 6-keto-PGF$_{1\alpha}$ release for 0.1, 0.3, and 0.9 mg/kg, respectively. Inhibition of prostanooid release was statistically significant for all doses tested (P < .001 for each dose and prostanooid). Meloxicam inhibited PGE$_2$ and 6-keto-PGF$_{1\alpha}$ release somewhat more potently (66.1 ± 9.0, 71.5 ± 25.8, and 78.6 ± 16.6 and 61.7 ± 17.4, 68.2 ± 20.1, and 75.5 ± 17.0, respectively) than diclofenac (40.0 ± 10.3, 49.2 ± 15.9, and 60.4 ± 6.8% and 20.3 ± 12.1, 28.4 ± 8.0, and 59.0 ± 19.8%, respectively). The differences between the two drugs at the respective doses (1, 3, and 9 mg/kg), however,
were not statistically significant. Compared with vehicle each meloxicam dose significantly reduced PGE$_2$ and 6-keto-PGF$_{1 \alpha}$ release ($P < .001$ for each dose and prostanoid, respectively). For diclofenac, PGE$_2$ release was significantly reduced at 1 ($P = .045$), 3 ($P = .003$) and 9 mg/kg ($P < .001$) but only the highest dose tested (9 mg/kg) led to a statistically significant reduction of 6-keto-PGF$_{1 \alpha}$ release ($P < .001$). With NS-398 PGE$_2$ and 6-keto-PGF$_{1 \alpha}$ release was modestly affected leading to a ~20 to 25% reduction of prostanoid release compared with vehicle. The difference to the control group was not statistically significant.

**Lung.** After administration of vehicle, fragments of rat lung released $542.7 \pm 148.9$ pg 6-keto-PGF$_{1 \alpha}$/mg wet weight/10 min and $145.5 \pm 68.4$ pg TXB$_2$/mg wet weight/10 min. In addition, leukotriene release was determined for the lung. After vehicle, $1.1 \pm 0.5$ pg LTB$_4$/mg wet weight/10 min and $0.8 \pm 0.5$ pg LTC$_4$/mg wet weight/10 min were released. The percentage inhibition of 6-keto-PGF$_{1 \alpha}$ and TXB$_2$ release from the lungs is shown in Fig. 3, A and B, respectively and the percentage increase of LTB$_4$ and LTC$_4$ release is shown in Fig. 4, A and B, respectively. Ketorolac inhibited 6-keto-PGF$_{1 \alpha}$ ($66.0 \pm 10.6$, $84.2 \pm 7.9$, and $84.2 \pm 10.5\%$) and TXB$_2$ ($76.9 \pm 8.7$, $91.9 \pm 6.2$, and $91.8 \pm 6.9\%$) release more potently than the other drugs tested. Inhibition of 6-keto-PGF$_{1 \alpha}$ and TXB$_2$ release by ketorolac was statistically significant for all doses tested ($P < .001$ for all doses and both prostanoids). Ketorolac led to a significant increase of LTB$_4$ ($133.6 \pm 19.5, P = .004, 95\%$ CI for differences 57.3–211.2%) and LTC$_4$ release ($152.3 \pm 164.5\%, 95\%$ CI for differences 36.7–261.3%) at 0.9 mg/kg compared with vehicle. The release of 6-keto-PGF$_{1 \alpha}$ and TXB$_2$ were almost equally reduced by meloxicam and diclofenac at the respective doses, leading to a maximum 6-keto-PGF$_{1 \alpha}$ inhibition of $43.6 \pm 11.9$ and $57.8 \pm 21.2\%$ and a maximum TXB$_2$ inhibition of $67.9 \pm 9.9$ and $54.1 \pm 27.9\%$ for meloxicam and diclofenac, respectively. Statistically, TXB$_2$ release after administration of diclofenac and meloxicam was significantly reduced at each dose tested ($P < .001$ for each drug and dose). The release of 6-keto-PGF$_{1 \alpha}$ differed significantly from vehicle for 1, 3, and 9 mg/kg meloxicam ($P = .02$ for 1 mg/kg and $P < .001$ for 3 and 9 mg/kg) and for 3 and 9 mg/kg diclofenac ($P < .001$). LTB$_4$ and LTC$_4$ release were not statistically significantly influenced.
by meloxicam or diclofenac, respectively. NS-398 did not show an alteration of prostanoid or leukotriene release from lung fragments at the doses tested.

**Brain.** After administration of vehicle, fragments of rat brain released 2.4 ± 0.9 pg PGF$_{2\alpha}$/mg wet weight/10 min and 3.1 ± 0.7 pg TXB$_2$/mg wet weight/10 min incubated ex vivo (Fig. 5, A and B). As for the other organs, ketorolac inhibited PGF$_{2\alpha}$ and TXB$_2$ release more potently than meloxicam, diclofenac, and NS-398. Maximum inhibition of prostanoid release by ketorolac (PGF$_{2\alpha}$, 61.0 ± 9.6%; TXB$_2$, 80.6 ± 9.8%) was somewhat less than for the other organs. Nevertheless, inhibition of TXB$_2$ and PGF$_{2\alpha}$ release was statistically significant compared with vehicle for the three doses tested (TXB$_2$, $P < .001$ for all doses; PGF$_{2\alpha}$, $P = .003$ for 0.1 mg/kg and $P < .001$ for 0.3 and 0.9 mg/kg, respectively). Meloxicam and diclofenac led to a dose-dependent 30 to 50% and 20 to 40% inhibition of TXB$_2$ release, respectively. PGF$_{2\alpha}$ release was inhibited by 25 to 30% and 50 to 60% by meloxicam and diclofenac, respectively. There was no statistically significant difference between both drugs at the doses tested. However, the effects of meloxicam and diclofenac on TXB$_2$ and PGF$_{2\alpha}$ release significantly differed from vehicle (TXB$_2$-diclofenac, $P < .001$ for 3 and 9 mg/kg; TXB$_2$-meloxicam, $P < .001$ each dose; PGF$_{2\alpha}$-diclofenac, $P < .001$ each dose; and PGF$_{2\alpha}$-meloxicam, $P = .038$ for 1 mg/kg, $P = .007$ for 3 mg/kg, and $P = .005$ for 9 mg/kg). NS-398 inhibited PGF$_{2\alpha}$ release by 27.7 ± 16.7 and 28.4 ± 13.7% at 3 and 9 mg/kg, respectively. The difference compared with vehicle was statistically significant for both doses ($P = .03$, 95% CI for differences 1.0 to 54.3% and $P = .009$, 95% CI for differences 3.5 to 53.3% for 3 and 9 mg/kg, respectively).

**Discussion**

The choice of drugs in the present study was based on ratios of COX-1/2 inhibition reported in various in vitro studies. Although these selectivity ratios are extremely dependent on the concentration of drug and substrate used in the assays, most workers would see NS-398 as a COX-2-selective agent (IC$_{50}$ COX-1/IC$_{50}$ COX-2 > 100) (Futaki et al.,

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**Fig. 4.** Percentage increase (mean ± S.D.) of LTB$_4$ and LTC$_4$ release from the lung after oral administration of 0.1, 0.3, and 0.9 mg/kg ketorolac (○) and 1, 3, and 9 mg/kg of each of diclofenac (●), meloxicam (□), and NS-398 (■). *P < .05, **P < .01, and ***P < .001.

**Fig. 5.** Dose-response curves for inhibition of PGF$_{2\alpha}$ and TXB$_2$ release from rat brain fragments ex vivo 60 min after oral administration of 0.1, 0.3, and 0.9 mg/kg ketorolac (○) and 1, 3, and 9 mg/kg of each of diclofenac (●), meloxicam (□), and NS-398 (■). Data show percentage inhibition (mean ± S.D.) of PGF$_{2\alpha}$ and TXB$_2$ release compared with vehicle-treated control rats. *P < .05, **P < .01, and ***P < .001.
1994; Ogino et al., 1997; Patrignani et al., 1997), whereas the selectivity ratios of ketorolac, meloxicam and diclofenac are all close to 1 (range 0.1–10) and the differences between them in terms of COX selectivity minor (Patrignani et al., 1997; Cryer and Feldman, 1998; Giuliano and Warner, 1999; Warner et al., 1999). The toxicity of ketorolac, however, is much higher than that of diclofenac and meloxicam, which may be related to its higher potency or to some drug-specific prostaglandin-independent mechanisms that were not addressed in the present study. Because the principal known mechanism of NSAID toxicity, however, is the inhibition of prostaglandin synthesis, the observed effects of prostanoid release provide some insight into the organ-specific toxicity of the respective drugs.

It has been shown previously that COX-2 is constitutively expressed in brain and spinal cord (Yamagata et al., 1993; Breder and Saper, 1996; Beiche et al., 1998) and to a lesser extent in renal inner medulla and renal cortex (Harris et al., 1994; Yang et al., 1999). Consistent with this tissue distribution, the selective COX-2 inhibitor NS-398 was found to
significantly inhibit PGF$_{2\alpha}$ release from rat brain by ~30% and to produce a slight reduction of PGE$_{2}$ and 6keto-PGF$_{1\alpha}$ release from the kidney. PGF$_{2\alpha}$ release from rat brain was similarly reduced by diclofenac, whereas meloxicam and ketorolac reached a maximum of ~60% inhibition. Because COX-2 is the predominant isoform in the brain (Yamagata et al., 1993; Breder and Saper, 1996; Yang et al., 1999), only minor differences between the drugs were expected. However, brain uptake may vary between drugs and may contribute to differences in the inhibitory properties.

In the kidney, COX-2 was reported to be localized to the macula densa and surrounding cortical thick ascending limb and its expression was found to increase in high-renin states, such as salt restriction and angiotensin-converting enzyme inhibition. (Harris et al., 1994; Yang et al., 1999). It was therefore suggested that COX-2-derived prostaglandins are important in the regulation of salt, volume, and blood pressure homeostasis (Harris et al., 1994). Although PGE$_{2}$ and 6keto-PGF$_{1\alpha}$ release was not significantly affected by NS-398 in our rats, COX-2 inhibition may become more important in hypertension or sodium and water loss. In salt-depleted subjects, the selective COX-2 inhibitor celecoxib caused sodium and potassium retention (Rossat et al., 1999), suggesting that increased selectivity for COX-2 does not spare the kidney, at least during salt depletion.

Unexpectedly, NS-398 reduced 6keto-PGF$_{1\alpha}$ release from rat gastric mucosa in a dose-dependent manner. The doses administered in the present study have been shown previously to be COX-2-selective (Ogino et al., 1997; Wallace et al., 1998). COX-2 expression in the healthy stomach is very low (O’Neill and Ford-Hutchinson, 1993; Mizuno et al., 1997; Sawaoka et al., 1997; Yang et al., 1999). Similarly, COX-2 protein is not constitutively expressed in the lung and as expected, NS-398 had no effect on 6keto-PGF$_{1\alpha}$ and TXB$_{2}$ release. Assuming that NS-398 is equally distributed to the stomach and lung, it appears unlikely that the drug lost its COX-2 selectivity in one organ but not in the other. However, because drugs were administered orally it cannot be excluded that higher local concentrations occurred in the stomach.

It has been shown recently that COX-2 mRNA and protein are strongly induced in gastric tissue of mice in which an ulcer had been induced. Treatment with NS-398 resulted in a reduction of mucosal prostaglandin synthesis and significant inhibition of ulcer healing (Mizuno et al., 1997). Similarly, treatment with the COX-2-selective inhibitor L745,337 resulted in marked inhibition of gastric ulcer healing in rats (Schmaassmann, 1998) at doses previously shown to produce selective inhibition of COX-2 (Chan et al., 1995). In addition, COX-1 knockout mice showed no gastric pathology and had less indomethacin-induced gastric ulceration than wild-type mice (Langenbach et al., 1995). These results suggest that COX-2 accounts for an important portion of prostaglandin synthesis in the rat stomach at least when there is some sort of gastric damage. It has been reported that COX-2 can be rapidly induced even in response to a minor mucosal irritation such as oral administration of acetylsalicylic acid (Davies et al., 1997; Sawaoka et al., 1997). Oral administration of the mild irritant 20% ethanol protected the gastric mucosa against the injury induced by subsequent instillation of 70 or 96% ethanol. Pretreatment of rats with COX-2-selective agents at doses required for anti-inflammatory effects reduced this adaptive protective response of the stomach (Gretzer et al., 1998). It is possible that in our experimental setup, COX-2 was up-regulated during fasting or by oral administration of the drugs. Indeed, with immunohistochemistry we observed a COX-2 expression in the stomach of rats who had received an i.p. injection of 0.9% saline solution, which can be regarded as a mild stress (Fig. 6).

Some growth factors were shown to induce COX-2 expression in gastric epithelial cells (Sasaki et al., 1998) and NS-398 inhibited hepatocyte growth factor-mediated gastric epithelial restitution (Horie-Sakata et al., 1998), suggesting that COX-2-derived prostaglandins contribute to maintaining mucosal integrity. Clinical studies, however, have demonstrated that the incidence of gastrointestinal ulcers with COX-2-selective inhibitors is comparable with that of placebo (Simon et al., 1998) and a recent in vitro study comparing the actions of >40 NSAIDs clearly supports the theory that inhibition of COX-1 underlies the gastrointestinal toxicity of NSAIDs in humans. This may imply that COX-2 plays a more important role in the rat stomach compared with the human stomach. In contrast, it has been shown that “adaptive cytoprotection” is a physiological phenomenon in humans, too, that appears to depend at least in part on the endogenous prostaglandin production (Foschi et al., 1986).

In conclusion, we have shown that the COX-2 selective inhibitor NS-398 significantly inhibits 6keto-PGF$_{1\alpha}$ release from rat gastric mucosa at doses that have been shown previously to be COX-2 selective. COX-2, therefore, appears to contribute to prostaglandin synthesis in the rat stomach at least after mucosal irritation which might have been caused by fasting or oral administration of the drug.

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