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RESEARCH PAPER

Effects of a non-selective COX inhibitor and selective COX-2 inhibitors on contractility of human and porcine ureters *in vitro* and *in vivo*

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Background and purpose: Anti-inflammatory drugs are used in the treatment of acute renal colic. The aim of this study was to investigate the effects of selective COX-2 inhibitors and the non-selective COX inhibitor diclofenac on contractility of human and porcine ureters *in vitro* and *in vivo*, respectively. COX-1 and COX-2 receptors were identified in human ureter and kidney.
Experimental approach: Human ureter samples were used alongside an *in vivo* pig model with or without partial ureteral obstruction. COX-1 and COX-2 receptors were located in human ureters by immunohistochemistry.
Key results: Diclofenac and valdecoxib significantly decreased the amplitude of electrically-stimulated contractions in human ureters *in vitro*, the maximal effect (V_{max}) being 120 and 14%, respectively. Valdecoxib was more potent in proximal specimens of human ureter ($EC_{50} = 7.3 \times 10^{-11}$ M) than in distal specimens ($EC_{50} = 7.4 \times 10^{-10}$ M), and the V_{max} was more marked in distal specimens (22.5%) than in proximal specimens (8.0%) *in vitro*. In the *in vivo* pig model, parecoxib, when compared to the effect of its solvent, significantly decreased the maximal amplitude of contractions (A_{max}) in non-obstructed ureters but not in obstructed ureters. Diclofenac had no effect on spontaneous contractions of porcine ureter *in vivo*. COX-1 and COX-2 receptors were found to be expressed in proximal and distal human ureter and in tubulus epithelia of the kidney.
Conclusions and implications: Selective COX-2 inhibitors decrease the contractility of non-obstructed, but not obstructed, ureters of the pig *in vivo*, but have a minimal effect on electrically-induced contractions of human ureters *in vitro.*
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Keywords: COX-2; COX-1; ureter peristalsis; ureteric calculi

Abbreviations: EFS, electrical field stimulation; NSAID, non-steroidal anti-inflammatory drug; PBS, phosphate-buffered saline

Introduction

COXs are enzyme complexes that catalyse the formation of prostanoids from arachidonic acid. To date, two distinct isoenzymes, COX-1 and COX-2, which differ in their expression pattern and regulation, have been identified (Habenicht *et al*., 1985; Herschman, 1996). Enzymatic activities of these COX isoforms produce prostanoids that play a pro-inflammatory role by mediating fever, hyperalgesia and vasodilatation. COX-1 is expressed in most tissues and is regarded as a constitutive enzyme involved in physiological functions such as mucus secretion in the stomach (Ikari *et al*., 1999), whereas COX-2 is inducible (Sirois and Richards, 1993; Crofford *et al*., 1997; Herschman *et al*., 1997). However, COX-2 has been reported to be constitutively expressed in certain tissues such as the kidney (Harris *et al*., 1994; Ferreri *et al*., 1999), brain and spinal cord (Breder *et al*., 1995). Non-steroidal anti-inflammatory drugs (NSAIDs) can alter renal function by reducing glomerular filtration rate, renal blood flow, and sodium and potassium excretion (Catella-Lawson *et al*., 1999). Both COX enzymes are known to be present in the kidney and their related prostanoids are involved in the regulation of fluid balance and blood pressure (Warner and Mitchell, 2002). An increase in COX-2 expression has been demonstrated in ureteral obstruction in the rat (Norregaard *et al*., 2006), and prostanoids have also been found to increase the contractility of electrically-stimulated human ureters (Cole *et al*., 1988).

Recently, a number of inhibitors relatively selective for COX-1 and COX-2 have been discovered, each of them demonstrating differing degrees of potency in their ability to inhibit COX activity (Riendeau *et al*., 2001). In addition, inhibition of COX-1 has been found to cause intestinal hypermotility, bacterial invasion, inducible NOS expression, and upregulation of COX-2 (Tanaka *et al*., 2002).

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Acute ureteral colic resulting from ureteral obstruction is a common and painful event in urolithiasis. Pharmacological relaxation of the ureter smooth muscle would facilitate the treatment of ureter stone colic as well as prepare the ureter for easier endoscopic access. Smooth muscle relaxant drugs may reduce smooth muscle contraction and spasm on the level of the stone, causing obstruction and increase of the intraluminal pressure, which is finally responsible for the severe colic pain.

NSAIDs, such as diclofenac, are used to treat patients suffering from renal colic caused by ureteric calculi, symptomatically. These compounds have been demonstrated to have anti-inflammatory and analgesic effects, whereas little is known about the possible effects of NSAIDs on ureteral smooth muscle. Indomethacin has been shown to reduce the frequency of severe colic attacks (Grenabo and Holmlund, 1984), whereas diclofenac, despite its analgesic effect, does not improve stone passage rate in renal colic (Kapoor *et al*., 1989; Laerum *et al*., 1995).

However, diclofenac has been shown to inhibit the spontaneous activity of sheep ureters (Thulesius *et al*., 1987), to cause relaxation of KCl-contracted human ureters (Sivrikaya *et al*., 2003), and of porcine 5-hydroxytryptamine-stimulated ureter segments (Mastrangelo *et al*., 2000). Furthermore, diclofenac has been shown to almost abolish the contractile response of electrically-stimulated human ureter muscle *in vitro* (Cole *et al*., 1988), and to induce a faster release of ureteric calculi and reduce the pain of patients suffering from renal colic (Hetherington and Philp, 1986).

Although the effects of COX-2 inhibitors on the contractility of ureteral smooth muscle of several species have been evaluated (Mastrangelo *et al*., 2000; Jerde *et al*., 2005), little information on their effects on human ureter are available in the literature until recently. Hence, the aim of this study was to investigate the expression of both COX-1 and COX-2 receptors in the human ureter and kidney. An *in vivo* model for partial ureteral obstruction was developed in piglets so that the effects of selective COX-2 inhibitors and non-selective inhibitors on ureteral contractility could be evaluated in samples of human ureters, *in vitro*, and porcine ureters, *in vivo*. As prostanoids have been shown to increase the contractility of human ureters (Cole *et al*., 1988), it was hypothesized that inhibitors of COX-1 and COX-2 would decrease the contractility of these ureters.

Methods

Immunohistochemistry

Sample processing. Histology showed that no tumour cells were present in the samples used for immunohistochemistry. Ten ureters (five proximal and five distal samples from five female and five male patients) were collected for immunohistochemistry and samples from four kidneys were used to investigate the expression pattern of COX-1 and COX-2 receptors. All samples were processed immediately after surgery.

Kidneys were cut transversally. Two specimens of about $1\,cm^3$ were excised and rinsed in phosphate-buffered saline (PBS). One sample was fixed in 0.5% glutaraldehyde with 4% paraformaldehyde, whereas the other was fixed in 4% paraformaldehyde in PBS, both for 12 h. Each sample included all the different regions of the kidneys, that is, cortex, medulla and pelvis. Ureters were prepared as described for the *in vitro* experiments and placed in fixatives for 6 h. Subsequently, tissue samples were embedded in paraffin. Sections of about 7 μm in thickness were mounted on APES-coated glass slides. Paraffin sections were dewaxed and immunostained as summarized in Table 1. Mouse anti-COX-1 and rabbit anti-COX-2 were used as specific primary antibodies. They were detected with corresponding peroxidase-conjugated secondary antibodies, 3,3′diaminobenzidine tetrahydrochloride being used as a chromogen. For control reactions, rabbit anti-calcitonin was used as an irrelevant primary antibody.

Slides were semiquantitatively analysed using a Zeiss microscope (Axioskop 2; Carl-Zeiss AG, Feldbach, Switzerland) equipped with a digital high-resolution camera (Axiocam HR; Carl-Zeiss AG). Labelling was graded as absent (−), weak (+), moderate (++) or strong (+++).

In vitro *experiments*

Materials. Kidney and ureter samples were obtained from patients undergoing partial or total nephrectomy and cystectomy, respectively, at the Department of Urology, University of Berne (Berne, Switzerland). Kidney samples were obtained from three male patients and one female patient with a mean age of 45 ± 10.4 years, whereas ureter samples were collected from patients ($n = 11$; seven male and four female patients) with a mean age of 66.2 ± 2.1 years. Specimens from proximal ($n = 5$) or distal ($n = 6$) human ureters were used for the *in vitro* experiments. The proximal ureter is defined as that from the ureteropelvic junction to the height of the pelvic bone; the distal ureter is defined as that part from about 7–10 cm cranially of the ureteral orifice to the ureteral orifice. Ureter tissue was obtained from

Table 1 Incubation protocol for COX-1 and COX-2 receptor antibodies

Steps	*Reagents*	*Duration*
Antigen retrieval	Citrate buffer	120 min
Washing steps	PBS	2 × 5 min
Quenching	3% H_2O_2 in methanol (COX-1)	30 min
Washing steps	PBS	2 × 5 min
First antibody	Mouse anti-COX-1 IgG 1:100 in PBS containing 5% normal goat serum	Overnight
	Rabbit anti-COX-2 IgG 1:250 in PBS containing 5% normal goat serum and 0.5% casein	Overnight
Washing steps	PBS	2 × 5 min
Secondary antibody	Goat anti-mouse and goat anti-rabbit IgG, peroxidase labelled (COX-1)	30 min
	Goat anti-rabbit IgG, peroxidase labelled (COX-2)	30 min
Washing steps	PBS	2 × 5 min
DAB reaction	DAB with 0.1% H_2O_2 (COX-1)	6–8 min
	DAB in imidazole buffer (COX-2)	10–15 min
Washing steps	PBS	2 × 5 min
	Distilled water	2 min

Abbreviations: DAB, 3,3′-diaminobenzidine; IgG, immunoglobulin G; PBS, phosphate-buffered saline.

patients undergoing nephrectomy or cystectomy. All tissue specimens appeared macroscopically normal with no sign of tumour or inflammation. The patients did not receive NSAIDs 3 days prior to surgery.

Measurement of ureteral contractility. Adjacent tissue was removed from the ureter tissue and rings of 0.3 cm length were suspended in the organ bath as described previously (Weiss *et al.*, 2002). Samples were immediately placed in Krebs–Henseleit solution at 4 °C, and the specimens were maintained in the solution at this temperature during transportation and sample processing.

Samples were placed in 70 mL organ baths with Krebs–Henseleit solution at 37 °C and gassed with 95% CO_2 and 5% O_2. The initial tension was set at 0.8 g, which had been found to be optimal in previous experiments (Weiss *et al.*, 2002), and electrical field stimulation (EFS) started after 15 min equilibration. As human ureteral tissue displays no or only little spontaneous peristaltic activity (Hertle and Nawrath, 1985), EFS was applied. EFS responses of human ureter have been shown previously to be insensitive to TTX (Cole *et al.*, 1988; de Moura and de Lemos, 1996). EFS was started after 5 min of equilibration with two platinum electrodes placed parallel to the ureter specimens. The stimulation pattern consisted of trains of 300 ms at an interval of 20 s and impulses of 125 mA with a duration of 5 ms at a frequency of 50 Hz applied by a Grass stimulator (Grass S88 stimulator; Grass Instruments, Quincy, MA, USA). Isometric forces were recorded using a force transducer type 351 (Hugo Sachs Electroniks, March, Germany). The mechanical response of the specimens was amplified and recorded on a personal computer, using the data acquisition system Power Lab (ADInstruments, Spechbach, Germany).

Only samples displaying contractions with regular frequency and amplitude in the predrug period were considered for this study. One hour after the beginning of EFS, cumulative concentration–response curves were constructed and compounds were added to the organ bath every 5 min. Diclofenac was used at final bath concentrations of 10^{-9}–10^{-5} M and valdecoxib at bath concentrations of 10^{-11}–10^{-6} M. Solvent control experiments were performed under the same conditions using ureter tissue from the same ureter sample as for the COX inhibitor experiment.

The following parameters were analysed: mean amplitude of contractions (A_{mean}), maximal amplitude of contractions (A_{max}), frequency and basal tone. The parameters were calculated at 5 min intervals for each preparation and concentration separately, using the software Chart included in the Power Lab system (ADInstruments).

In vivo *experiments*

Animals and surgery. The *in vivo* experiments were performed on a total of 21 female 'large white' pigs with a mean body weight of 21.64 ± 0.32 kg ($n = 7$ for diclofenac; $n = 8$ for parecoxib; $n = 6$ for control animals (solvent)). All animals in this study received care according to the Laws on Care and Use of Laboratory Animals in Switzerland. Experiments were approved by the Bernese State Committee for Animal Experimentation.

Premedication was performed by administration of ketamine (10 mg kg^{-1} i.m.) followed by xylazine (2 mg kg^{-1} i.m.) and thiopental (8 mg kg^{-1} i.v.) with atropine (0.05 mg kg^{-1} i.v.). Anaesthesia was maintained using isoflurane in an oxygen–nitrogen mix (ratio 1:1). Continuous i.v. infusion was performed with 0.5 mL h^{-1} saline solution. Hydroxyethyl starch was used to stabilize blood pressure above 60 mm Hg.

Experimental procedure. As previously described by Danuser *et al.* (2001), one catheter was inserted into the carotid artery to measure arterial blood pressure and blood gases, and another catheter was placed into the jugular vein to administer infusions and drugs. A double lumen 6-french catheter was inserted through the animal's lateral abdominal wall into each renal pelvis. One lumen was placed in the renal pelvis allowing perfusion of upper urinary tract; the other lumen was placed in the proximal to midportion of the ureter to measure intraluminal pressure. In addition, another double lumen 6-french catheter was inserted in each distal ureter just at the junction into the bladder. The bladder was catheterized to drain off urine.

To establish a partial ureteral obstruction, one of the distal double lumen catheters was connected with an external water column of 15 cm height. This partial obstruction was performed 30 min before the first administration of the compounds in one ureter, whereas the contralateral ureter remained unobstructed. Effects of compounds were determined in obstructed ureters compared with non-obstructed ureters in the same animal and compared with solvent controls. Animals without spontaneous ureter contractility were excluded from this study. Contractions in ureters, arterial blood pressure and heart rate (ECG) were recorded using a Hellige recording system (SMU 611, Freiburg, Germany).

Solvent (0.9% NaCl) or increasing doses of the COX-2 inhibitor parecoxib, and the non-selective COX inhibitor diclofenac were given i.v. via the catheter placed in the jugular vein. Parecoxib was administered every 35 min, because this compound undergoes metabolism in the liver to the active compound valdecoxib. Diclofenac and solvent (eight times 2 mL, for control animals) were administered every 10 min. Diclofenac was administered at doses ranging from 0.01 to 10.0 mg kg^{-1} and parecoxib at doses ranging from 0.001 to 3.0 mg kg^{-1}.

Data analysis

All values were computed as percentage of control results and given as mean ± s.e.mean or confidence limits. Data were statistically analysed using ANOVA for repeated measures followed by the Bonferroni's multiple comparison test with the software Number Cruncher Statistical Systems 97 (NCSS2000; Kaysville, Utah, USA) and Minitab 14 (Minitab Inc., State College, PA, USA). A P-value <0.05 was considered significant.

Concentration–response curves were calculated using the Hill's equation and estimation by use of least squares method using the MatLab Simulation Software (the Mathworks Inc. 2002, Natick, MA, USA).

The underlying equation for the Hill's function is

$$\text{Response} = V_{max} C^{\alpha}(C^{\alpha} + K^{\alpha})^{-1}$$

where V_{max} is the maximal attainable response, C is the compound concentration, K stands for the EC_{50}, and the exponent α describes the shape of the function (Hill's coefficients with values greater than 1 describe curves with a flat low-dose region and high curvature, whereas values smaller than 1 correspond to curves that climb rapidly). Statistical significance of comparisons made on the basis of this model was determined using the likelihood ratio statistic, which yields a χ^2 test. The results are expressed as V_{max} and EC_{50} of BT, A_{max} and A_{mean}, respectively.

Drugs and reagents

Diclofenac sodium salt (2-[(2,6-dichlorophenyl]amino) benzeneacetic acid sodium salt; Sigma/Fluka, Buchs, Switzerland); valdecoxib (4-(5-methyl-3-phenyl-4-isoxazolyl) benzenesulphonamide; Pfizer, New York, NY, USA). Distilled water was used as a solvent for diclofenac and DMSO (dimethyl sulphoxide GR dried; Dr Grogg AG, Stettlen, Switzerland) was used as solvent for valdecoxib with the final DMSO ratio in the organ bath being 0.013%.

Krebs–Henseleit solution consisted of (mmol L^{-1}) 118.4 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 25 $NaHCO_3$ and 11 glucose (Uchida *et al*., 1994), all salts were from Dr Grogg AG.

The drugs used were Diclofenac (Voltaren; Novartis, Basel, Switzerland); parecoxib (predrug of valdecoxib, *N*-[[4-(5-methyl-3-phenylisoxazol-4-yl)phenyl]sulphonyl]proponamide; Pfizer). Drugs were diluted in sterile NaCl (0.9%) to obtain the final dilution.

The reagents used were PBS (PBS Tablets; Calbiochem, Dr Grogg AG; 10 mM phosphate buffer, pH 7.4, 140 mM NaCl and 3 mM KCl); citrate buffer (ChemMate Target Retrieval Solution (× 10); DakoCytomation, Carpontrei, USA); mouse anti-ovine COX-1 monoclonal IgG and rabbit anti-murine COX-2 polyclonal IgG (Cayman Chemical Company, Ann Arbor, MI, USA); rabbit anti-calcitonin antibody (Anawa Trading SA, Wangen, Switzerland); normal goat serum and casein sodium salt from bovine milk (Sigma/Fluka); goat anti-rabbit peroxidase-labelled IgG (EnVision; DakoCytomation) and goat anti-mouse and anti-rabbit peroxidase-labelled IgG (EnVision DualLink, DakoCytomation); DAB (3,3′-diaminobenzidine; Fluka); imidazole buffer (0.15 M NaCl and imidazole from Dr Grogg AG, and 0.05 M tris(hydroxymethyl)aminomethane from Sigma); fixative (0.5% glutaraldehyde, 4% paraformaldehyde, 50 mM lysine-HCl from Dr Grogg AG in 0.1 M cacodylate buffer, pH 7.4); and APES (3-aminopropyltrieethoxysilane; Sigma).

The anaesthetic isoflurane in an oxygen–nitrogen mix (ratio 1:1) was obtained from Fabius GS (Dräger Medical, Telford, USA). Saline solution was used for i.v. infusion (Ringer-Lactate; Fresenius Kabi, Stans, Switzerland) and hydroxyethyl starch (Voluven; Fresenius Kabi, Stans, Switzerland).

Results

Expression of COX-1 and COX-2 receptors

Receptors for both COX-1 and COX-2 were detected in human ureters by immunohistochemistry. Receptors were found to be localized to the urothelium, to smooth muscle cells in the tunica muscularis of ureters and to the tunica media of blood vessels (small arterioles).

Distribution of COX-1 receptors was similar in proximal and distal regions of the ureter. Receptors were shown to be present in the superficial layers of the urothelium and in the perinuclear region of smooth muscle cells (see Figures 1c and e).

In contrast, COX-2 receptors were evenly distributed in all the strata of the urothelium. The perinuclear region of smooth muscle cells of the tunica muscularis of ureters and of blood vessels was labelled as well (see Figures 1d and f). No differences in the locations of the signals were noted between proximal and distal regions of the ureter.

Negative controls in which the specific primary antibody had been substituted with an anti-calcitonin antibody were devoid of any signal as shown in Figures 1g and h.

Kidney samples, used as positive control tissues, displayed strong immunoreactivity in tubulus epithelium. However, interstitial tissue and the glomeruli remained unstained, as shown in Figures 1a and b.

Effects of diclofenac and valdecoxib on electrically-stimulated human ureters in vitro

Figure 2 illustrates the effect of diclofenac (Figure 2a) and valdecoxib (Figure 2b) on the A_{mean} of samples of human ureter *in vitro*.

Administration of the non-selective COX inhibitor diclofenac resulted in a significant ($P<0.05$) and concentration-dependent decrease in A_{mean} (49.14 ± 5.42%), when compared with predrug values (Figure 2a). A significant ($P<0.05$) inhibition of the A_{max} (58.73 ± 6.56%) was also observed. The effect was significantly different from the solvent control for both parameters. A decrease in contractility, compared with predrug, was seen in samples from the proximal ureter ($A_{mean} = 43.10 \pm 11.99\%$; $A_{max} = 45.96 \pm 11.59\%$) and in distal specimens ($A_{mean} = 53.3 \pm 4.77\%$; $A_{max} = 67.24 \pm 6.25\%$). A significant difference in the V_{max} for A_{mean} was obtained when proximal samples were compared with distal tissue samples (see Figure 3a and Table 2). Diclofenac was shown to be more potent in proximal samples when compared with distal samples (Table 2).

Valdecoxib inhibited A_{mean} and A_{max} with the effect being statistically significant ($P<0.05$) only at the highest concentration (10^{-6} M) when compared with predrug values, as shown in Figure 2b. In contrast to diclofenac, a significant difference ($P<0.05$) in A_{mean} was estimated for valdecoxib when proximal samples were compared with distal samples, as illustrated in Figure 3b and Table 2. No significant differences in any of the parameters investigated were observed when the results with valdecoxib were compared with those of the solvent control.

The maximal effect of diclofenac was more marked than that of valdecoxib; the V_{max} values being 120.8 and 14.4%,

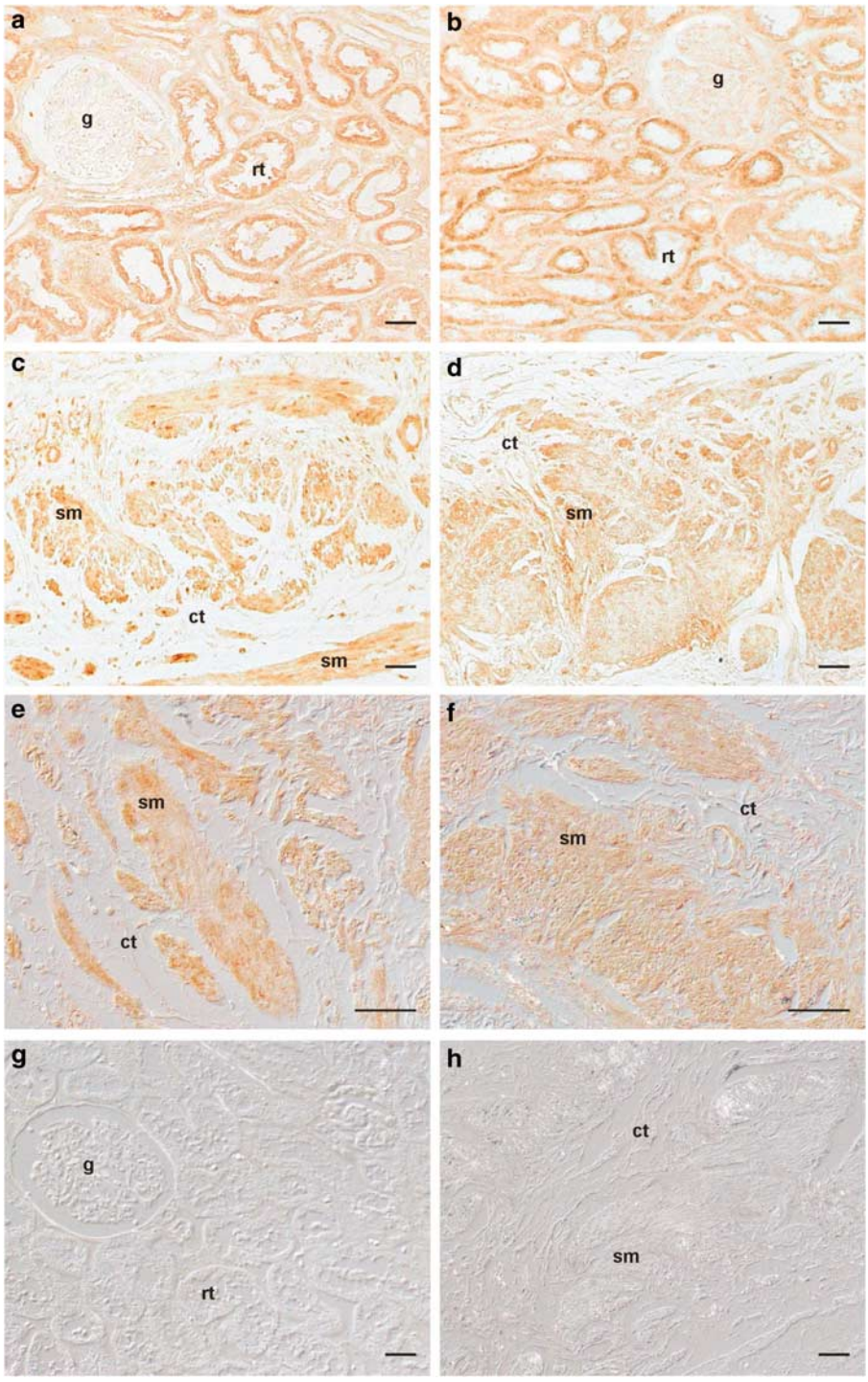

Figure 1 Localization of COX-1 and COX-2 on human kidneys and ureters with indirect immunoperoxidase labelling on paraffin wax sections. DAB (3,3′-diaminobenzidine) was used as a chromogen. Renal cortex specimens incubated with anti-COX-1 (**a**), with anti-COX-2 (**b**), and with anti-calcitonin as an irrelevant substitute for specific primary antibodies (**g**). In kidneys, a strong immunoreactivity was observed in renal tubule epithelium. Ureter specimens incubated with anti-COX-1 (**c**, **e**), with anti-COX-2 (**d**, **f**) and with anti-calcitonin as an irrelevant substitute for specific primary antibodies (**h**). Receptors for COX-1 and COX-2 are localized to smooth muscle cells in the tunica muscularis of ureters. Bright field (**a**–**d**) and differential interference contrast (**e**–**h**). g = glomerulum, rt = renal tubule, sm = smooth muscle cells, ct = connective tissue. Scale bar = 50 μm.

respectively ($P<0.001$) as shown in Table 2 and Figure 3c. However, from the concentrations investigated in this study, valdecoxib was estimated to be more potent than diclofenac ($EC_{50} = 1.8 \times 10^{-10}$ M (valdecoxib) and 2.5×10^{-6} M (diclofenac), $P<0.001$) (Table 2). The EC_{50} value for diclofenac might be an underestimate of the true value because the curve did not reach a plateau.

As shown in Figure 2, the effects of these compounds on contractility parameters were not reversible by flushing of the organ bath with Krebs–Henseleit solution.

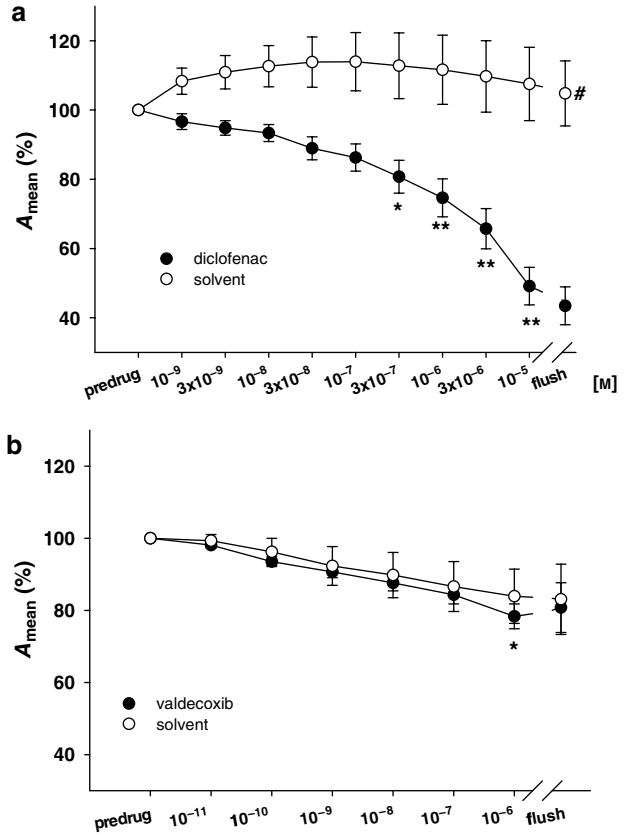

Figure 2 Concentration–response curves for the effects of diclofenac (**a**) and valdecoxib (**b**) on the mean amplitude of contractions (A_{mean}) in specimens from human ureter ($n=11$) *in vitro*. Compounds were added in a cumulative manner at 5 min intervals. Values are shown relative to predrug and given as mean ± s.e.mean. Significant differences from predrug are given as asterisks (*$P<0.05$; **$P<0.01$); differences between curves are shown as $^{\#}P<0.005$.

Effects of diclofenac and parecoxib on porcine ureter contractility in vivo

Ureteral contractility parameters remained constant in all experiments during the predrug period. No significant changes in blood pressure, heart rate and blood gases were observed during the experiment. One pig without spontaneous ureter contractility was excluded from this study.

Comparison of partially obstructed ureters versus non-obstructed ureters. A model for partial ureter obstruction was established and effects of solvent were recorded over time. Interestingly, on comparing the partially obstructed with the non-obstructed ureters, a significant difference in amplitude of contractions was observed under physiological conditions (solvent = 0.9%, NaCl), as shown in Figure 4. An increase in A_{max} was obtained in non-obstructed ureters, whereas a decrease in A_{max} was seen in partially obstructed ureters. No difference between these models was observed for frequency of contractions and basal tone.

Effects of parecoxib in vivo. Parecoxib induced a dose-dependent decrease in A_{max} in non-obstructed porcine ureter *in vivo* when compared with solvent, as shown in Figure 5a,

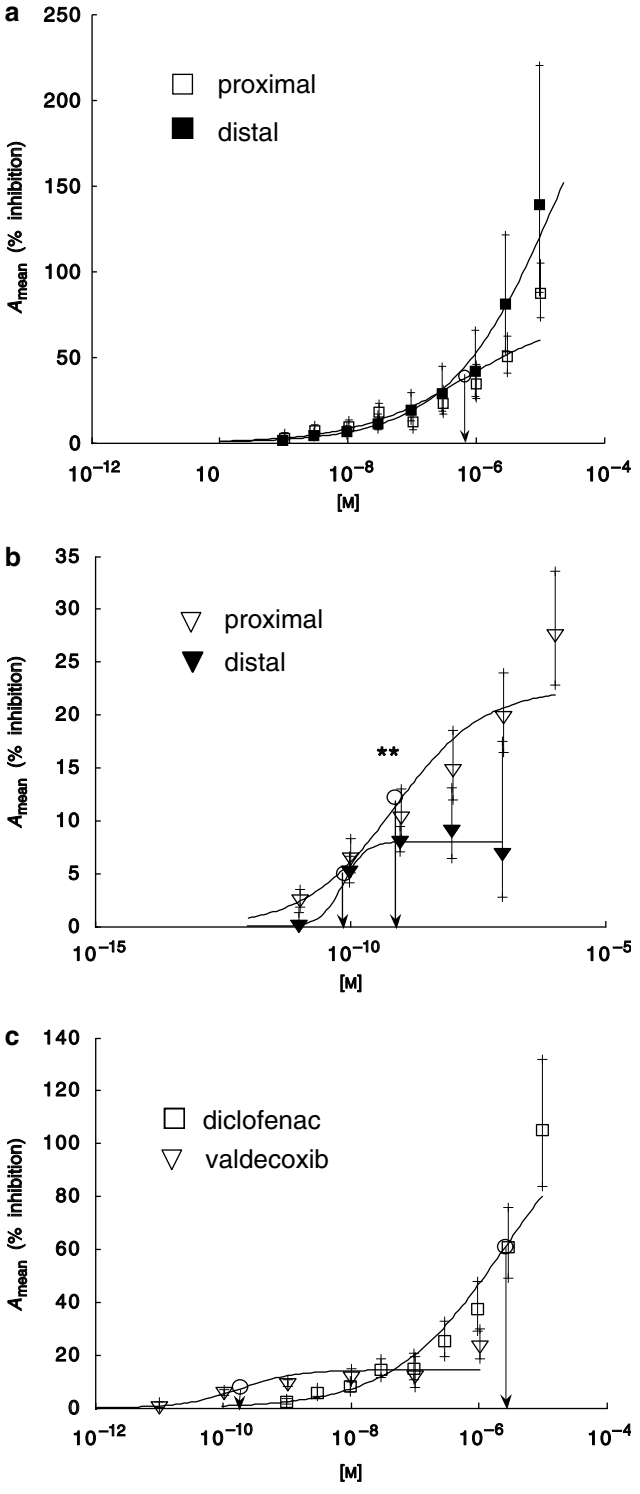

Figure 3 The effect of diclofenac (**a**) or valdecoxib (**b**) on the mean amplitude of contractions (A_{mean}) is shown for proximal ($n=5$) and distal ($n=6$) specimens from human ureter. Effects of valdecoxib and diclofenac on A_{mean} *in vitro* (**c**). Arrows indicate EC_{50} values. Significant differences between both locations for valdecoxib are given as asterisks (**$P<0.01$).

whereas no effect on this parameter was seen in partially obstructed ureters (Figure 5b). In partially obstructed ureters, a significant decrease in the frequency of contractions,

Table 2 Effect of diclofenac and valdecoxib on the mean amplitude of contractions in human ureter specimens *in vitro*

Compound location	n	V_{max} *(%)*	*Lower CL (%)*	*Upper CL (%)*	EC_{50} *(M)*	*Lower CL (M)*	*Upper CL (M)*
Diclofenac							
All	11	120.75[a]	28.52	511.16	2.5×10^{-6b}	1.28×10^{-8}	0.0004
Proximal	5	300	8.46	10641	2.2×10^{-10c}	7.75×10^{-10}	0.63
Distal	6	75.42	26.34	215.93	6.63×10^{-7c}	7.46×10^{-9}	5.89×10^{-5}
Valdecoxib							
All	11	14.36[a]	9.89	20.84	1.8×10^{-10c}	4.13×10^{-11}	8.01×10^{-10}
Proximal	5	8.03[d]	4.08	15.841	7.23×10^{-11d}	1.30×10^{-11}	4.07×10^{-10}
Distal	6	22.47[d]	9.11	55.43	7.37×10^{-10d}	3.00×10^{-12}	1.81×10^{-7}

Abbreviations: CL, 95% confidence limits; EC_{50}, effective concentration 50% (M); V_{max}, maximal obtainable effect (% of inhibition); .
Values with the same subscript letter are significantly different from each other (a, b and d, $P<0.001$; c, $P<0.05$).

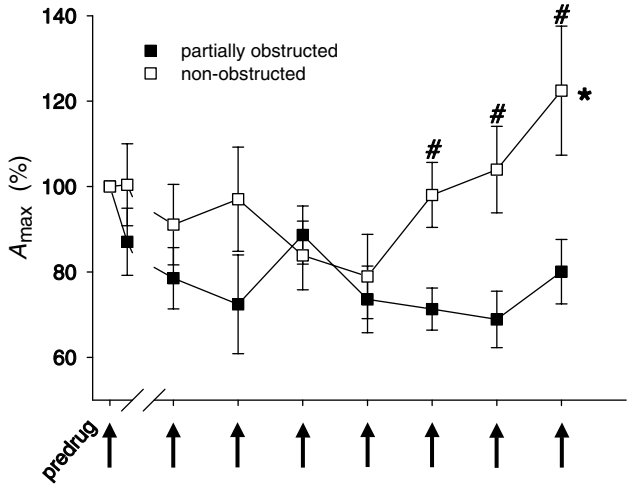

Figure 4 Comparison of ureter models *in vivo*. Effect of solvent (NaCl) on maximal amplitude of contractions (A_{max}) in ureters with and without partial obstruction. Arrows indicate i.v. administration of 2 mL solvent ($n=8$). Values are expressed relative to predrug and data are given as mean ± s.e.mean. A_{max} was measured intraureteral using a double lumen 6-french catheter, as described in Methods. A significant difference between both curves, partially obstructed ureter versus non-obstructed ureter, is denoted as $^{*}P<0.05$. A comparison of the effect of each dose with that of the predrug effect was performed and statistically significant effects are denoted as $^{\#}P<0.05$.

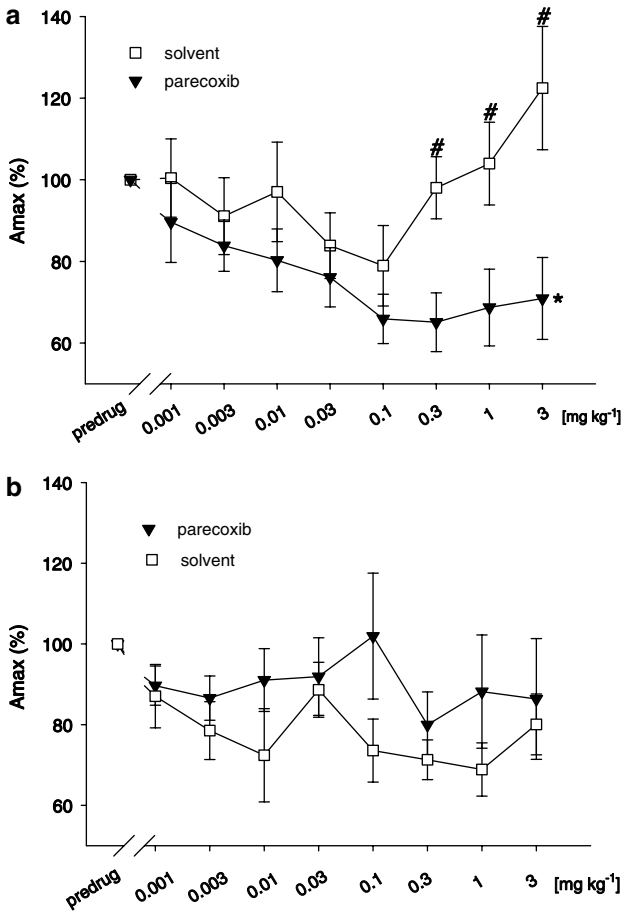

Figure 5 Dose–response curves for the effects of parecoxib and solvent on the maximal amplitude of contractions (A_{max}) in non-obstructed ureters (**a**) and partially obstructed ureters (**b**) of piglets ($n=8$) *in vivo*. Values are mean ± s.e.mean. Administration (i.v.) of parecoxib was performed every 35 min in a cumulative manner (0.001–3 mg kg^{-1}). A_{max} was measured intraureteral using a double lumen 6-french catheter, as described in Methods. A significant difference between dose–response curves for parecoxib and solvent is denoted as $^{*}P<0.05$. A comparison of the effect of each dose with that of the corresponding administration of solvent was performed and statistically significant effects are denoted as $^{\#}P<0.05$.

compared with those in the presence of the solvent, was seen with 1 and 3 mg kg^{-1} parecoxib. A decrease in basal tone was also seen at the highest doses investigated (0.3, 1 and 3 mg kg^{-1}). Interestingly, no significant difference in these parameters was seen in the non-obstructed ureter. The V_{max} and ED_{50} values are given in Table 3.

In non-obstructed ureters, a dose-dependent increase in basal tone (Figure 6a) and frequency was obtained in the presence of parecoxib, whereas a decrease in these parameters was seen in partially obstructed ureters (but this effect on basal tone was not significant when compared with the predrug value). A model comparison revealed a significant effect ($P<0.05$) of parecoxib on both A_{max} and basal tone in non-obstructed ureters (Figure 6a; Table 3).

Effects of diclofenac in vivo. In the presence of diclofenac, a significant difference ($P<0.05$) was calculated for the basal tone in non-obstructed ureters compared with partially obstructed ureters (Figure 6b), whereas diclofenac had no effect on the values for A_{max} and frequency. Diclofenac had no significant and/or dose-dependent effects on A_{max}, frequency and basal tone in non-obstructed ureters when

Table 3 Effect of parecoxib on smooth muscle contractility in piglets *in vivo*

Model/parameter	V_{max} *(%)*	*Lower CL (%)*	*Upper CL (%)*	ED_{50} *(mg kg^{-1})*	*Lower CL (mg kg^{-1})*	*Upper CL (mg kg^{-1})*
Non-obstructed ureters						
Frequency	88.46	17.51	446.84	0.07	0.0005	9.94
A_{max}	50.82	0.64	4007.3	0.07	0.0001	43.57
BT	183.67	34.91	966.41	0.001	2.11×10^{-8}	47.28
Partially obstructed ureters						
BT	60	22.44	160.42	0.006	3.65×10^{-5}	1.09

Abbreviations: A_{max}, maximal amplitude; BT, basal tone; V_{max}, maximal obtainable effect (%). A decrease in all parameters was seen for partially obstructed ureter, whereas an increase in BT was seen in non-obstructed ureters. CL = 95% confidence limits; ED_{50}, effective dose 50% (mg kg^{-1}).
No values for V_{max} and ED_{50} could be obtained for A_{max} and frequency in partially obstructed ureters.

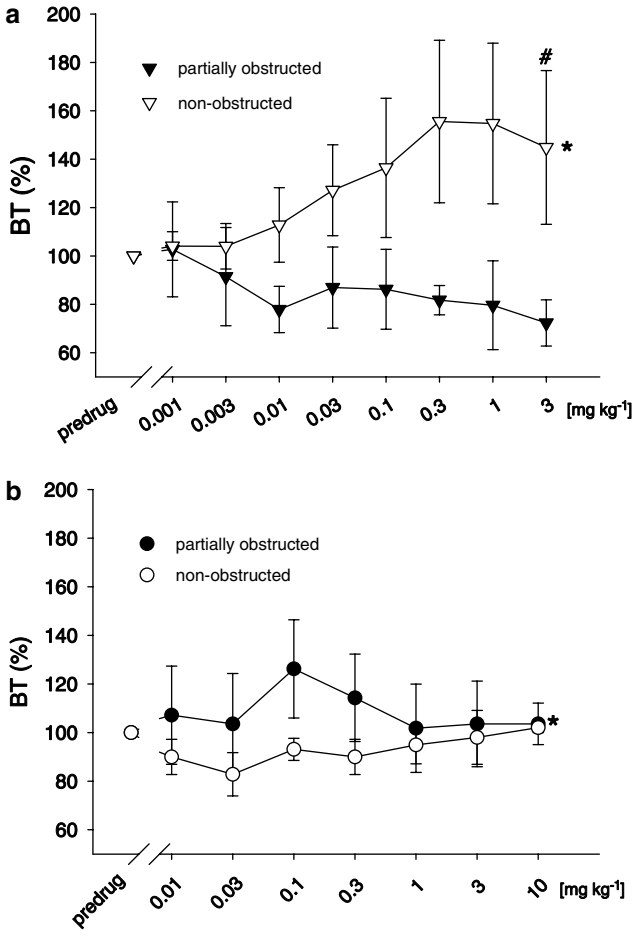

Figure 6 Effect of partial ureteral obstruction on basal tone (BT) in piglets treated with parecoxib (**a**) ($n = 8$) or diclofenac (**b**) ($n = 7$) *in vivo*. Administration (i.v.) of compounds was performed in a cumulative manner. Diclofenac was administered every 10 min (0.01–10 mg kg^{-1}) and parecoxib was given every 35 min (0.001–3 mg kg^{-1}). Parameters were measured intraureteral using a double lumen 6-french catheter, as described in Methods. Significant differences between both models (partially obstructed ureter versus non-obstructed ureter) are denoted as $^{*}P < 0.05$. A comparison of the effect of each dose with that of the corresponding administration of solvent was performed and statistically significant effects are denoted as $^{\#}P < 0.05$.

the results obtained were compared with predrug values. The effect of diclofenac on A_{max} is shown in Figure 7a. In partially obstructed ureters, diclofenac had a significant effect on A_{max} ($P < 0.05$) when compared with that of the solvent (Figure 7b). Diclofenac significantly decreased the basal tone observed, when compared with solvent, at the highest dose (10 mg kg^{-1}) investigated, but had no effect on the frequency of contractions when compared with predrug or solvent values.

Discussion

In the present study, we demonstrated that COX-1 and COX-2 receptor proteins are expressed in urothelium, smooth muscle cells and in the tunica muscularis and the tunica media of blood vessels (small arterioles) of human ureter. Furthermore, COX-2 was present in renal tubule and macula densa. In kidneys, COX-1 and COX-2 were observed in renal tubule epithelium. Our results on the functional effects of the selective COX-2 inhibitor, valdecoxib, and the non-selective COX inhibitor, diclofenac, demonstrate that diclofenac induces a concentration-dependent decrease in the amplitude of contractions in electrically-stimulated human ureters *in vitro*. Valdecoxib had no effect on ureter contractility when compared with solvent.

A significant increase in the amplitude of contractions was seen over time in porcine non-obstructed ureters compared with partially obstructed ureters *in vivo*. In comparison to the *in vitro* data, a dose-dependent effect of the non-selective COX inhibitor, diclofenac, on ureteral contractility was not obtained in pig ureters *in vivo*. The COX-2 inhibitor, parecoxib, reduced the amplitude of ureteral contractions in non-obstructed ureters, but had no effect in the partially obstructed porcine ureters.

Our immunohistochemistry results are in agreement with those obtained by Fornai *et al*. (2005). This group demonstrated that COX-1 and COX-2 are constitutively expressed in colonic tunica muscularis (Fornai *et al*., 2005). Also, our findings on the intracellular localization of both COX proteins in human ureters are consistent with the results of Murakami *et al*. (2003). These authors demonstrated that both COX-1 and COX-2 are located in the perinuclear envelope, and the ER membrane, with COX-1 being dispersed into the cytoplasm along the ER membrane and COX-2 being more prevalent in the perinuclear area in HEK293 cells.

In rodent kidney, Campean *et al*. (2003) demonstrated that COX-1 is located in mesangial cells of the glomerulus, in terminal distal tubule, in connecting tubule and cortical, and medullary collecting ducts. In our study, COX-2 was found

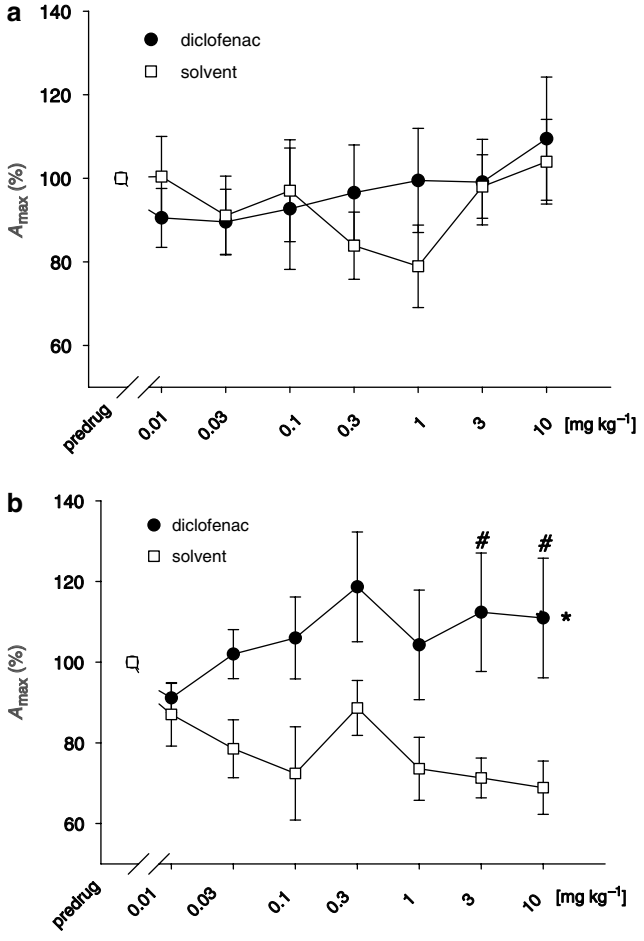

Figure 7 Dose–response curves for the effect of diclofenac compared with that of solvent on maximal amplitude (A_{max}) in non-obstructed ureters (**a**) and partially obstructed ureters (**b**) from piglets ($n=7$) *in vivo*. Values are mean ± s.e.mean. Diclofenac was administered (i.v.) every 10 min in a cumulative manner (0.01–10 mg kg^{-1}). Parameters were measured intraureteral using a double lumen 6-french catheter, as described in Methods. A significant difference between diclofenac and solvent is denoted as $^{*}P<0.05$. A comparison of the effect of each dose with that of the corresponding administration of solvent was performed and statistically significant effects are denoted as $^{\#}P<0.05$.

to be present in renal tubule and macula densa. Furthermore, staining in vascular smooth muscle cells was absent. These differences could be due to species differences. In contrast to our findings, in humans, COX-1 has been shown to be expressed in collecting duct cells, interstitial, endothelial and smooth muscle cells of pre- and postglomerular vessels, whereas COX-2 is localized in endothelial and smooth muscle cells of arteries, veins and intraglomerular regions of podocytes (Komhoff *et al*., 1997). In adult humans, COX-2 was shown to be associated with the endothelium of vasa recta and medullary capillaries, whereas COX-1 is only localized in collecting ducts and macula densa.

Nakada *et al*. (2002) demonstrated that COX-2 mRNA and protein levels are upregulated in chronically obstructed human ureters. We used non-obstructed ureter samples, and both COX-1 and COX-2 proteins were detected in samples from human ureter.

NSAIDs are currently considered a first-line treatment of renal colic. Their action has been ascribed to the inhibition of renal prostaglandin synthesis, which decreases renal blood flow and diuresis, and consequently lowers the pressure in the renal pelvis and ureter.

Diclofenac and the selective COX-2 inhibitor NS-398 have been shown to be almost equipotent in reducing neurokinin-A-induced contractions in the porcine isolated ureter *in vitro* (Mastrangelo *et al*., 2000). We also demonstrated that diclofenac decreases the contractility of human ureters *in vitro*. However, in our study, valdecoxib did not decrease the amplitude of contractions of human ureters when compared with solvent controls. This inconsistency might be due to different methods of stimulation of the ureteral specimens (EFS versus neurokinin-A- or 5-HT-induced contractions) or the fact that Mastrangelo *et al*. (2000) used a different selective COX-2 compound (valdecoxib versus NS-398). In addition, our experiments were performed in human ureter samples, whereas Mastrangelo *et al*. (2000) used porcine samples.

Indomethacin has been demonstrated to cause a concentration-dependent inhibition and/or suppression in EFS-evoked contractions in guinea-pig ureter, but these effects were obtained at high concentrations (100–500 μM) (Santicioli *et al*., 1995). Indomethacin and NS-398 completely abolished the frequency of contractions in human and porcine ureters *in vitro* (Nakada *et al*., 2000). Furthermore, celecoxib, a selective COX-2 inhibitor, indomethacin and NS-398 have been demonstrated to inhibit TNFα-induced ureteral contractions in porcine ureter *in vitro* (Jerde *et al*., 2005). However, the effect of these COX inhibitors was investigated on spontaneous ureteral contractions occurring at a frequency of 5–10 contractions per 5 min. Again, different methodological parameters might explain the differences in the results. Specimens were suspended in the organ bath in a longitudinal manner, whereas we used ureter rings. However, Jerde *et al*. (1999) did ascertain that no differences in contractions were evident when ureter rings were compared with spiral cut and longitudinal segments (Jerde *et al*., 1999). Furthermore, in their study, the effect of only one concentration of each compound was investigated, whereas we constructed concentration–response curves. Inhibition of COX-1 has also been shown to increase the amplitude and the frequency of contractions in rat ureter but to have no effect in the guinea-pig ureter (Davidson and Lang, 2000). Davidson and Lang (2000) also showed that NS-398, a selective COX-2 inhibitor, reduced the motility index (amplitude × frequency) in the guinea-pig upper urinary tract but had no effect on this variable in rat upper urinary tract.

COX inhibitors have been found to cause smooth muscle relaxation in a variety of smooth muscle tissues. In contrast to our data on ureteral smooth muscle, diclofenac has been demonstrated to inhibit distension-induced rhythmic contractions in rat urinary bladder *in vivo*, and this effect was found to be proportional to its effectiveness as an anti-inflammatory agent (Maggi *et al*., 1984). From these results, it was suggested that arachidonic acid metabolites could regulate micturition by enhancing the amplitude of myogenic contractions in the bladder, but no analysis of prostaglandin synthesis was performed in this study.

Indomethacin and NS-398 have been shown to decrease contractility in guinea-pig trachea *in vitro* (Charette *et al*., 1995). COX-1 sparing drugs have been reported to exert tocolysis in human myometrium *in vitro*, and this effect was less marked when non-selective COX inhibitors were used (Slattery *et al*., 2001). Indomethacin has also been shown to inhibit myometrial contractility via mechanisms independent of COX (Sawdy *et al*., 1998). In guinea-pig isolated small intestine, SC-560, a selective COX-1 compound, and NS-398 did not affect contractility *in vitro*, whereas indomethacin disturbed the regular pattern of propulsive motility in this species by an effect unrelated to COX inhibition (Shahbazian *et al*., 2001). Therefore, differences in function of COX inhibition occur within species but also smooth muscle tissues. Different expression levels within tissues and/or species may explain the inconsistent effects of selective COX-2 inhibitors on smooth muscle contractility.

In our *in vivo* model, an acute partial obstruction was induced about 30 min before the compounds were administered to healthy piglets. A significant difference in A_{max} was obtained between partially obstructed and non-obstructed ureters under physiological conditions. Therefore, the model used to investigate the effects of compounds is important with respect to clinical consequences or conclusions. COX-2 expression has been shown to be upregulated in chronically obstructed human (Nakada *et al*., 2002) and porcine ureters (Jerde *et al*., 2006) *in vivo*.

In our study, diclofenac decreased the contractility of pig ureters *in vitro* in a concentration-dependent manner, whereas no dose-dependent effect of diclofenac on contractility was seen *in vivo*. Recent findings from Davenport *et al*. (2007) support this finding; they demonstrated that diclofenac had no effect on the contraction frequency in the human ureter *in vivo*. In our study, the selective COX-2 inhibitor, parecoxib reduced the amplitude of contractions in porcine non-obstructed ureters when compared with solvent, whereas no effect on contractility was seen in partially obstructed ureters. Parecoxib has been shown to attenuate the increase in pelvic pressure that occurs during obstruction in rats *in vivo*, but the dose used in that study was considerably higher than those used in our study (Norregaard *et al*., 2006). Also, Norregaard *et al*. (2006) found an increased expression of COX-2 protein in dilated ureter compared with non-dilated ureter suggesting that COX-2 activity contributes to increased pressure after obstruction. Furthermore, urinary prostaglandin-E_2 excretion was increased after release of the obstruction. Interestingly, inhibition of prostanoid synthesis has been found to reduce ureteral contractility rates *in vivo* and *in vitro*, in support of the decrease in contractility seen with parecoxib in non-obstructed ureters. However, it has recently been demonstrated that prostaglandin-E_2 increases contractility in chronically obstructed ureters but inhibits contractility in non-obstructed ureters (Lowry *et al*., 2005).

In the *in vitro* studies, differences in harvesting, localization, storage, preparation and experimental design could explain the different results reported. In the *in vivo* studies, small alterations in the renal pelvis can affect peristaltic activity in the ureter and the smooth muscle is particularly susceptible to physical interferences (Ancill *et al*., 1972). Drugs used for general anaesthesia might also cause relaxant effects on the ureter (Young *et al*., 1994), and, therefore, the *in vivo* results may not represent the true effects of the COX inhibitors.

In addition to the analgesia and anti-inflammatory potential of COX-2 inhibitors (Mehlisch *et al*., 2003), a relaxant effect on the ureter might also be beneficial in patients with acute stone colic. Ureteric peristalsis has always been assumed to be essential for the spontaneous passage of stones, and our *in vivo* findings showed that diclofenac did not affect ureter contractility in pigs. In acute ureteral colic resulting from ureteral obstruction, pharmacological relaxation of the ureter smooth muscle would facilitate the treatment of ureter stone colic as well as prepare the ureter for easier endoscopic access. Whereas our *in vitro* findings suggest that diclofenac has potential spasmolytic properties and might be beneficial with respect to treatment of ureter stone colic, the *in vivo* findings in pigs did not demonstrate an effect on ureteral contractility. Hence, as we demonstrated that COX-2 inhibitors have no effect on the contractility of human isolated ureters or of porcine partially obstructed ureters *in vivo*, apart from their known analgesic effect, it is unlikely that they would be useful in the treatment of ureteric stone colic.

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Conflict of interest

The authors state no conflict of interest.

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