Modulation by stereoselective inhibition of cyclo-oxygenase of electromechanical coupling in the guinea-pig isolated renal pelvis

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1 The effects of the (S)- and (R)-enantiomers of the cyclo-oxygenase (COX) inhibitor, ketoprofen, have been investigated on the spontaneous activity of the guinea-pig isolated renal pelvis and on electrical field stimulation-(EFS) induced contractions of the guinea-pig ureter in comparison with the effects of the achiral COX inhibitor, indomethacin.

2 (S)-ketoprofen (0.1–100 μM) produced a concentration- and time-dependent inhibition of the spontaneous myogenic activity of the renal pelvis. The maximal inhibitory effect (% inhibition of motility index) averaged 29, 42, 47 and 56% inhibition of control values at 0.1, 1, 10 and 100 μM . The (R)-enantiomer was ineffective up to 10 μM .

3 Indomethacin (0.1–100 μM) likewise produced a concentration- and time-dependent inhibition of spontaneous motility of the isolated renal pelvis: its maximal inhibitory effect was larger than that produced by (S)-ketoprofen and averaged 21, 40, 69 and 95% inhibition of motility index at 0.1, 1, 10 and 100 μM respectively. In the presence of a maximally effective (100 μM) concentration of (S)-ketoprofen, 100 μM indomethacin produced >90% inhibition of residual motility.

4 In the guinea-pig isolated ureter, phasic contractions were induced by EFS (5 ms pulse width, 60 V): (S)-ketoprofen (100–500 μM) had no effect on the EFS-evoked contractions. Indomethacin (100–500 μM) produced a concentration-dependent inhibition and/or suppression of the EFS-evoked contractions. When contraction of the ureter was evoked by 80 mM KCl, indomethacin produced about 30 and 80% inhibition at 100 and 300 μM , respectively, while (S)-ketoprofen (300 μM) was ineffective.

5 The effect of (S)-ketoprofen or indomethacin (10 μM each) on the propagation of myogenic impulses along the ureter was determined by use of a three chamber organ bath. The renal end of the ureter was electrically stimulated while recording the mechanical activity of the renal and bladder ends of the ureter: addition of either (S)-ketoprofen or indomethacin (10 μM) did not effect propagation of impulses from the renal to the bladder end of the ureter, while nifedipine (10 μM) promptly blocked the propagated contractions.

6 In sucrose gap experiments, (S)-ketoprofen (10–100 μM) produced a time-dependent shortening of spontaneous action potentials of the guinea-pig renal pelvis and reduced the amplitude and duration of the accompanying phasic contractions. Indomethacin (10 μM) produced comparable effects on the same parameters and significantly reduced the maximal amplitude of depolarization of the pacemaker potential. In the presence of 100 μM (S)-ketoprofen, 100 μM indomethacin promptly suppressed the residual pacemaker potential and contraction.

7 Neither (S)-ketoprofen nor indomethacin (10 μM each for 60 min) affected the parameters of action potential and contraction of the guinea-pig ureter evoked by EFS. Both drugs produced a sustained membrane depolarization.

8 The present findings demonstrate that stereoselective COX inhibition affects pacemaker potentials and contractility (electromechanical coupling) in the guinea-pig renal pelvis. The modulatory role of endogenous prostanoids involves an amplification of electromechanical coupling in the renal pelvis while excitability, contractility or propagation of impulses along the ureter appear almost independent of prostanoid generation. Previous reports of a total suppression of pyeloureteral motility by indomethacin may reflect a combination of COX inhibition and nonspecific effect on electromechanical coupling.

Keywords: Guinea-pig ureter; guinea-pig renal pelvis; cyclo-oxygenase; ketoprofen; indomethacin; stereoselective

Introduction

Metabolites of arachidonic acid produced along the cyclo-oxygenase (COX) pathway (prostanoids) act as modulators/mediators of smooth muscle activity in the urinary tract. In the urinary bladder, prostanoids are produced during bladder distension at cystometry and exert both a direct myotropic action on detrusor muscle and a sensitizing activity on bladder afferents which set the gain for the activation of the

micturition reflex (Maggi, 1992, for review). A role of prostanoids in the local regulation of motility has also been postulated in the upper urinary tract but the picture emerging from the available data is far from clear (see below). The tissue is of clinical relevance because of the increasing evidence that COX inhibitors produce pain relief during renal colic (Holmlund & Sjodin, 1978; Lundstrom *et al.*, 1982; Oosterlink *et al.*, 1990).

Conflicting results have been reported with regard to the effect of prostaglandins on pyeloureteral motility. Prostaglan-

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dins of the E or F series produce excitatory effects on the motility of the ureter or renal pelvis (Thulesius & Angelo-Khattar, 1985; Lundstam *et al.*, 1985; Thulesius *et al.*, 1986; 1987; Cole *et al.*, 1988), but smooth muscle hyperpolarization, relaxation and adenosine 3': 5'-cyclic monophosphate (cyclic AMP) accumulation in response to added prostaglandins have been reported in some studies (Johns & Wooster, 1975; Vermue & Den Hertog, 1987). Since different prostanoids could be produced from the same tissue in a species- and stimulus-dependent manner, the results obtained with exogenously added prostaglandins do not necessarily reflect the physiological role of endogenous prostanoids, while the effects produced by COX inhibitors would be more informative in this respect.

Several groups have shown that COX inhibitors, for e.g. indomethacin, produce a profound inhibitory effect on spontaneous or evoked motility of isolated pyeloureteral smooth muscle from various species (Thulesius & Angelo-Khattar 1985; Lundstam *et al.*, 1985; Thulesius *et al.*, 1986; 1987; Cole *et al.*, 1988; Kimoto & Constantinou, 1991): the general conclusion emerging from these studies is that prostanoid generation is an important step for the local regulation of pyeloureteral motility. In some studies, a complete suppression of pyeloureteral motility by COX inhibitors has been reported (Lundstam *et al.*, 1984; Thulesius & Angelo-Khattar 1985; Thulesius *et al.*, 1987; Cole *et al.*, 1988): this may suggest that prostanoid generation is mandatory for the maintenance/activation of ureteral peristalsis. The evidence from this approach is, however, strictly limited by the selectivity and specificity of the pharmacological tools employed. Whenever high concentrations of COX inhibitors have been used for producing inhibition/total suppression of pyeloureteral motility (e.g. 10–100 μM indomethacin), the possibility cannot be completely ruled out that nonspecific drug effects on excitation/contraction coupling may have been produced. Indomethacin and other COX inhibitors have been reported to affect transmembrane ion movements in various cell types, including smooth muscle cells (Northover, 1971; 1973; Whittle, 1976).

A powerful tool to discriminate between specific and non-specific effects of drugs is stereoselectivity. In this study, we have employed the enantiomers of ketoprofen, a well known COX inhibitor, to assess the importance of endogenous prostanoids in regulating the spontaneous (renal pelvis) and evoked (ureter) motility of the guinea-pig isolated pyeloureteral tract. The action of the (S)-enantiomer of ketoprofen (active in blocking COX) and of the (R)-enantiomer (inactive in blocking COX) (Hayball *et al.*, 1992; Suesa *et al.*, 1993) were compared with that of indomethacin.

Methods

General

All experiments were performed on the renal pelvis or ureter excised from male albino guinea-pigs (250–300 g body wt) which were stunned and bled. The renal pelvis and ureter (from the inferior renal pole to its entry into the bladder) were excised, cleaned of adhering fat and connective tissue and placed in oxygenated and warmed Krebs solution (95% O_2 and 5% CO_2 , pH 7.4 at 37°C), as described previously (Maggi & Giuliani 1991; Maggi *et al.*, 1994a,b). The Krebs solution had the following composition (mmol^{-1}): NaCl 119, NaHCO_3 25, KH_2PO_4 1.2, MgSO_4 1.5, KCl 4.7, CaCl_2 2.5 and glucose 11.

Organ bath experiments

In a first series of experiments we investigated the effect of (S)- or (R)-ketoprofen and indomethacin on the spontaneous activity of the guinea-pig isolated renal pelvis: the renal pelvis

was opened, connected to silk threads and mounted in a 5 ml organ bath for isotonic recording (load 2 mN) of mechanical activity along its circular axis, as described previously (Maggi & Giuliani, 1992; Maggi *et al.*, 1994a). After setup, all preparations developed a spontaneous activity which reached steady state values of amplitude and frequency within 60 min. At this stage, the stated concentration of the drug under study was added to the bath and its effects were observed for 60 min. The inhibitory effects of COX inhibitors on the motility of the renal pelvis were evident either as a progressive reduction in the amplitude of contractions with a minor effect on their frequency or as a mixed inhibitory effect on both frequency and amplitude of contraction. As described previously (Maggi *et al.*, 1994a) a motility index (in arbitrary units) was calculated in each preparation by multiplying the frequency of spontaneous contractions (contraction min^{-1}) and their amplitude (in mm of chart); the inhibitory effect of drugs was expressed as % inhibition of the basal motility index.

In each preparation, only one concentration of test drug was investigated, except in experiments in which the additivity of the effect of (S)-ketoprofen and indomethacin were determined. (S)-ketoprofen and indomethacin were investigated at concentrations ranging between 0.1 and 100 μM . For (R)-ketoprofen, concentrations greater than 10 μM were not investigated: since the threshold concentration of (S)-ketoprofen is 0.1 μM (see Results) and the purity of the sample of (R)-ketoprofen used is 99.4%, the (R)-enantiomer if tested at concentrations > 10 μM would contain a fraction of (S)-enantiomer capable of exerting biological activity in the renal pelvis.

In a second series of experiments, we studied the effect of (R)- or (S)-ketoprofen toward electrically- or KCl-evoked contractions of the guinea-pig ureter. A segment of ureter about 2 cm long was excised from its middle portion and the specimen was mounted in a 5 ml organ bath for isotonic recording (load 3 mN) of mechanical activity along the longitudinal axis, as described previously (Maggi & Giuliani, 1991). The ureters were exposed to capsaicin 10 μM for 15 min to prevent the release of sensory neuropeptides produced by application of depolarizing stimuli, as described previously (Maggi *et al.*, 1994a,b). Electrical pulses of long width (5 ms, 60 V) were automatically delivered every 60 s by means of two platinum wire electrodes placed at the top and the bottom of the organ bath (field stimulation) by means of a GRASS S88 stimulator and in the presence of the calcium channel agonist, Bay K 8644 (1 μM). In separate experiments (Bay K 8644 was not added in this case) contractions of the ureter were elicited at 30 min intervals by application of KCl (80 mM, added to the bath for 10 min) and the effect of drugs determined after 25 min contact time.

Propagation of activity in the ureter

The following experiments were performed to assess whether COX inhibitors may affect propagation of impulses along the ureter: a method developed for this purpose was used. Briefly, male albino guinea-pigs weighing 250–300 g body wt. were stunned and bled. The whole kidney and ureter were excised and placed in a Petri dish containing oxygenated Krebs solution. A segment (4–5 cm in length) of ureter was dissected from the inferior renal pole and placed in a 3 chamber organ bath which enabled a separate superfusion of different parts of the organ. Two perspex partitions were used to separate the renal-, middle- and bladder sites (henceforth referred to as the R-, M- and B-site, respectively) which included a window covered with condom rubber: a small hole was made in the rubber to enable the passage of the ureter. Proximally to each partition, the renal and bladder ends of the ureter were pinned onto a Sylgard support. The renal and bladder ends of the ureter were connected via pulleys to isotonic transducers (Basile 7006) (load 2 mN) for recording of mechanical activity on a two-channel polygraph

(Basile 7080). Each compartment was perfused by means of a peristaltic pump at a rate of 1 ml min^{-1} with oxygenated (95% O_2 and 5% CO_2 , pH 7.4) Krebs solution at 34°C . All experiments were performed in ureters pre-exposed to capsaicin ($10 \mu\text{M}$ for 15 min).

Electrical field stimulation (EFS) was applied to the renal (R-site) compartment by means of two wire platinum electrodes positioned in parallel at the two sides of the ureter. Square wave pulses (5–25 ms pulse width, 20 V) were automatically delivered every 100 s by means of a GRASS S88 stimulator.

The method is based on the assumption that contractions recorded at the bladder end of the ureter (B-site) following application of depolarizing stimuli at the R-site depend upon propagation of action potentials throughout the ureter and that restricted drug application to the middle region (M-site) will affect propagation of impulses: the assumption was verified by experiments involving section of the ureter at the M-site, by comparing the effect of applying the stimulus at the R-, M- and B-sites and by studying the effect of nifedipine at each of the three compartments.

Sucrose gap experiments

Male albino guinea-pigs (250–300 g) were stunned and bled. The renal pelvis was excised and placed in oxygenated (95% O_2 and 5% CO_2) Krebs solution. A single sucrose-gap, modified as described in detail by Artemenko *et al.* (1982) and Hoyle (1987) was used to investigate changes in membrane potential and mechanical activity. The renal pelvis was superfused with oxygenated Krebs solution at a rate of 1 ml min^{-1} . The temperature of the solution was kept constant at $35 \pm 0.5^\circ\text{C}$. Under these conditions all preparations tested developed spontaneous electrical and mechanical activity within 10–20 min from setup. When the spontaneous activity had reached a steady state either (S)-ketoprofen (10–100 μM) or indomethacin (10–100 μM) was added to the superfusion medium and their effects were assessed over a 60 min observation period.

The duration of spontaneous (pacemaker) action potential and contraction were measured at 50% of repolarization and relaxation, respectively. The duration of repolarization was calculated from the peak of depolarization to 50% of repolarization.

In other experiments, the effects of (S)-ketoprofen or indomethacin (10 μM for 60 min) were investigated on the action potential and contraction produced by direct EFS in the guinea-pig ureter (Shuba, 1977; Brading *et al.*, 1983). The experiments were performed in capsaicin-pretreated ureters (10 μM for 15 min) and in the presence of Bay K 8644 (1 μM), as described previously (Maggi *et al.*, 1994a,b). Square wave pulses (1–2 ms pulse width, 10–20 V) were automatically delivered every 2.5 min by means of a GRASS S88 stimulator.

Statistical analysis

Each value is mean $\pm$ s.e.mean. Statistical analysis was performed by means of Student's *t* test or analysis of variance, when appropriate. Linear regression was performed by the least squares method: EC_{50} values and 95% confidence limits were calculated accordingly.

Drugs

Drugs used were: nifedipine, capsaicin and indomethacin (Sigma), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (Calbiochem). The (S)- and (R)-enantiomers of ketoprofen were synthesized in the Chemistry Department of Laboratories Menarini by enzymatic resolution of the racemic trifluoroethyl ester (Palomer *et al.*, 1993). The optical purity was determined by a direct enantiospecific h.p.l.c. method, using

a Chiralcel OJ column (Daicel, Japan), as described previously (Palomer *et al.*, 1993). The measured enantiomeric excess values were 99.4 and 99.7% for the (R)- and (S)-enantiomers, respectively.

Results

Organ bath

Effect on spontaneous activity of the renal pelvis The guinea-pig isolated renal pelvis shows spontaneous mechanical activity at a frequency of 4–7 contractions min^{-1} and an amplitude ranging between 40–65% of the maximal contractile response to added KCl. At steady state (about 60 min from setup), both frequency and amplitude of spontaneous contractions are stable for >60 min. The spontaneous activity of the renal pelvis was abolished within 90 min from the addition of 1 μM nifedipine ($n = 6$), indicating its close dependency from the activation of L-type voltage-sensitive calcium channels.

The addition of indomethacin or (S)-ketoprofen (0.1–100 μM) produced a concentration- and time-dependent inhibition of the spontaneous activity of the renal pelvis. The inhibitory effect was evident either as a reduction in the amplitude of contractions with only a minor effect on their frequency (as in the examples shown in Figure 1) or as a mixed inhibitory effect on both the amplitude and frequency of spontaneous contractions. To account for both effects, a motility index was calculated by multiplying the frequency and amplitude values before addition of drugs: the time-dependent changes of motility index produced by various concentrations of COX inhibitors are shown in Figure 2. At 0.1 and 1.0 μM , the maximal inhibitory effects of (S)-ketoprofen and indomethacin were comparable. At 10 and 100 μM the inhibitory effect of (S)-ketoprofen showed a plateau, whereby a substantial fraction (40–50% of control values) of motility index was preserved at 60 min from the addition of 100 μM (S)-ketoprofen.

On the other hand, 10–100 μM indomethacin produced a further marked and concentration-dependent inhibition of the motility index and in about 30% of cases tested, completely suppressed the spontaneous motility of the renal pelvis within 60 min of addition to the bath. On average, 10 and 100 μM indomethacin produced 68 ± 7 and $95 \pm 3\%$ inhibition of motility index at 60 min from its addition to the bath (Figure 2, $n = 6$ for each group). At both concentrations, the effect of indomethacin was significantly larger ($P < 0.05$) than that produced by (S)-ketoprofen (Figure 2).

Up to 10 μM , the (R)-enantiomer of ketoprofen did not significantly affect the motility index of the renal pelvis (Figures 1 and 3).

Since a substantial fraction of the spontaneous motility of the renal pelvis was unaffected by 100 μM (S)-ketoprofen, whilst indomethacin produced a much larger inhibitory effect, the effect of 100 μM indomethacin was also investigated on the (S)-ketoprofen-resistant (100 μM , 60 min before) activity of the renal pelvis. In these experiments, (S)-ketoprofen produced a $52 \pm 9\%$ inhibition of motility index ($n = 4$); the addition of 100 μM indomethacin produced a further marked inhibition or suppression ($90 \pm 2\%$ inhibition) of the residual activity.

Effect on stimulated contractions of the ureter Electrical field stimulation (EFS, 5 ms pulse width, 60 V every 60 s) produced phasic contractions of the guinea-pig ureter which, in the presence of Bay K 8644, were steady for several hours. Nifedipine produced 67 ± 8 and 100% inhibition of EFS-evoked contractions at 10 and 30 μM , respectively ($n = 6$). At 10 μM , neither (S)-ketoprofen nor indomethacin ($n = 4$ each) significantly affected the amplitude of EFS-evoked contractions of the ureter (Figure 3).

Figure 1 (a and b) Tracings showing the time dependent inhibitory effect produced by 100 μM (S)-ketoprofen and indomethacin on the spontaneous activity of the guinea-pig isolated renal pelvis. Note the much larger inhibitory effect produced by indomethacin as compared to (S)-ketoprofen. Vertical scales represent % of the maximal contractile response to KCl 80 mM. (c) Concentration and time-dependent inhibitory effect produced by cyclo-oxygenase (COX) inhibitors on motility of the guinea-pig isolated renal pelvis. In each preparation a motility index was calculated (in arbitrary units) by multiplying frequency and amplitude values of spontaneous contractions. The % inhibition of basal motility index produced by COX inhibitors is shown for (S)- and (R)-ketoprofen (left panel) or indomethacin (right panel) at various times after their addition to the bath. Concentrations tested are as follows: in left panel, (R)-ketoprofen 10 μM (○); (S)-ketoprofen 0.1 μM (●), 1 μM (■), 10 μM (▲) and 100 μM (◆); in right panel, indomethacin 0.1 μM (○), 1 μM (□), 10 μM (Δ) and 100 μM (◇). Each value is mean $\pm$ s.e.mean of 6–9 experiments. *Significantly different from control values, $P < 0.05$.

At 100–500 μM , indomethacin produced a concentration-dependent inhibition of the EFS-evoked contractions: the inhibitory effect developed quickly, each concentration producing steady state inhibition in 4–8 min; the effect averaged 13 ± 2 , 40 ± 5 and $82 \pm 6\%$ inhibition at 100, 300 and 500 μM , respectively ($n = 4$ for each concentration) and was rapidly reversed upon washout (Figure 3). (S)-ketoprofen was without significant inhibitory effect up to 500 μM ($n = 4$).

Application of KCl (80 mM for 5 min) produced a biphasic contraction of the guinea-pig ureter; both components of response are inhibited by $>90\%$ by 1 μM nifedipine after 120 min contact time ($n = 4$). Indomethacin produced 27 ± 9 and $72 \pm 6\%$ inhibition of the phasic response to KCl at 100 and 300 μM , respectively ($n = 4–6$). The corresponding values for the tonic component of the response to KCl were 29 ± 12 and $80 \pm 5\%$ inhibition, respectively. (S)-ketoprofen (100–300 μM , $n = 4$) had no effect on the responses to KCl.

Effect on propagation of impulses along the ureter Electrical field stimulation (EFS, 5 ms pulse width, 20 V) applied at the renal end (R-site) of the ureter produced a phasic contraction

at both the R- and bladder (B-site) ends of the ureter: both responses ranged between 40–60% of the response to KCl (80 mM) applied at the respective compartment and will be referred to hereafter as the direct and propagated responses to EFS, respectively. (S)-ketoprofen or indomethacin (10 μM each, $n = 4$) applied via superfusion at the M-site for 60 min did not affect either the direct or the propagated responses to EFS (Figure 4). In contrast, application of nifedipine (10 μM , $n = 4$) at the M-site promptly suppressed the propagated response without affecting the direct response to EFS (Figure 4).

Sucrose gap

Pacemaker potentials in the renal pelvis The guinea-pig renal pelvis showed a spontaneous electrical and mechanical activity at a mean frequency of 1.62 ± 0.11 cycles min^{-1} ($n = 54$) and an average amplitude of maximal depolarization and amplitude of contractions of 9.95 ± 0.47 mV and 4.84 ± 0.1 mN, respectively. Nifedipine (10 μM) produced a rapid suppression of spontaneous action potential and contractions of the guinea-pig renal pelvis (Figure 5).

The electrical activity of the guinea-pig isolated renal pelvis

Figure 2 Concentration-dependency of the inhibitory effect of (S)-ketoprofen (●) and indomethacin (○) on motility index of the guinea-pig isolated renal pelvis, measured as the maximal inhibitory effect observed within 60 min of drug addition to the bath. Note that the maximal effect of (S)-ketoprofen plateaued at about 50–60% inhibition while indomethacin produced an almost full blockade of spontaneous activity. Each value is mean $\pm$ s.e.mean of 6–9 experiments. *Significantly different from the inhibitory effect of (S)-ketoprofen, $P < 0.05$.

was characterized by a slow 'diastolic' depolarization or prepotential to a threshold value when a spontaneous (pacemaker) action potential was observed, followed by a phasic contraction. The shape of the action potential was quite heterogeneous from one preparation to another (see below), especially for the presence and duration of a plateau. In most instances (43 out of 54 cases tested) a series of small amplitude rapid fluctuations of membrane potential superimposed onto the ascending limb of the action potential and/or its peak (Figure 5 and 7). We noted that those preparations showing a pronounced plateau in the action potential also showed a relatively fast rate of depolarization.

Combining the two criteria, 2 groups of preparations were subjected to further analysis: group A was defined by a slower rate of depolarization ($< 15 \text{ mV s}^{-1}$) and absence of a pronounced plateau (Figure 5a); group B by a faster rate of depolarization ($> 15 \text{ mV s}^{-1}$) and presence of a plateau (Figure 5b). In the population of 54 preparations investigated, 9 cases were not classified because of their failure to meet one of the 2 above criteria, 22 cases were classified as group A and 23 cases as group B. The characteristics of the excitation-contraction cycle in Groups A and B are further analysed in Table 1. As can be seen, group A was characterized by a higher frequency of pacemaker potentials, lower values of maximal amplitude of depolarization, longer time to peak of depolarization and lower depolarization rate as compared to group B (Table 1). Furthermore, the time from peak depolarization to 50% repolarization was significantly shorter in group A than in group B, reflecting the absence of a pronounced plateau in the action potential in group A (Table 1). The amplitude of afterhyperpolarization was not significantly different in the two groups. The parameters of the contraction-relaxation cycle were likewise not different between group A and group B (Table 1).

The frequency of pacemaker potentials did not show significant changes over the 60 min observation period in either controls ($n = 13$, 5 from group A and 5 from group B), (S)-ketoprofen ($n = 11$, 4 from group A, 5 from group B) or indomethacin- ($n = 13$, 4 from group A and 4 from group B) treated groups. On average, there was a slight tendency to a

Figure 3 Tracings (a, b, c) and mean values (d) showing the effect of (S)-ketoprofen (○), indomethacin (□) and nifedipine (△) on EFS-induced contractions of the guinea-pig isolated ureter. In (d), each value is mean $\pm$ s.e.mean of 4–6 experiments. *Significantly different from control values $P < 0.05$. W = washout.

reduced frequency of pacemaker potentials in the control group (1.54 ± 0.24 and 1.39 ± 0.2 cycles min^{-1} before and after 60 min superfusion with drug-free Krebs solution, $n = 13$) and a slight tendency to an increased frequency in the indomethacin group (from 1.65 ± 0.29 to 1.89 ± 0.35 cycles min^{-1} , $n = 13$), while no change was evident in the (S)-ketoprofen-treated group (from 1.33 ± 0.13 to 1.38 ± 0.15 cycles min^{-1} , $n = 11$). In control preparations ($n = 13$) a slight decline in the maximal amplitude of depolarization was observed throughout the 60 min observation period (Figure 6): this small effect (16% reduction) was statistically significant at 50 and 60 min. Other parameters of the action potential and accompanying contraction did not show any significant time-related modification during a 60 min superfusion with drug-free Krebs solution (Figure 6).

Figure 4 Effect of indomethacin (a), (S)-ketoprofen (b) and nifedipine (c) on propagation of impulses along the guinea-pig ureter. The ureter was placed in a three-compartment organ bath and electrical stimuli were applied at its renal end while simultaneously recording mechanical activity at both renal and bladder ends (R- and B-sites, respectively). The response observed at the B-site is due to propagation of myogenic impulses along the ureter. Nifedipine applied at the middle compartment (M-site) suppressed the propagation of impulses while leaving the response recorded at the R-site unaffected. (S)-ketoprofen and indomethacin had no effect on the propagation of impulses along the guinea-pig ureter.

(S)-ketoprofen ($10 \mu\text{M}$, $n = 11$) and indomethacin ($10 \mu\text{M}$, $n = 13$) both produced a progressive, time-dependent modification of various parameters of the excitation contraction coupling of the guinea-pig renal pelvis (Figures 6 and 7a): these effects include a shortening of action potential duration (45 and 56% reduction by (S)-ketoprofen and indomethacin, respectively), a reduced amplitude of contraction (37 and 43% inhibition, respectively) and a shortening of contraction duration (36 and 47% inhibition, respectively). Indomethacin ($10 \mu\text{M}$) also produced a significant time-dependent reduction of the maximal amplitude of depolarization (32% inhibition at 60 min) which was significantly larger than the small spontaneous decline observed in controls (Figure 6). This effect of indomethacin was not shared by (S)-ketoprofen which produced a 9% reduction at 60 min (n.s. vs. time-matched controls, Figure 6).

Considering the differences in resting values of some parameters between groups A and B, a further analysis was made of possible differential effects of (S)-ketoprofen or

Figure 5 Examples (panels a and b) of the pacemaker potential recorded by sucrose gap from the guinea-pig renal pelvis smooth muscle. Preparations described in the text as group A (panel a, see also Table 1) are characterized by a relatively slow rate of depolarization and by the absence of a pronounced plateau; those described in the text as group B (panel b) are characterized by a relatively fast rate of depolarization and by a pronounced plateau in the action potential, similar to that recorded in the ureter. In both cases, small rapid oscillations of membrane potential were observed on the ascending limb of depolarization and/or on the peak of the action potential. (c) Nifedipine ($10 \mu\text{M}$) suppresses pacemaker potential (lower tracing) and spontaneous contractions (upper tracing) of the renal pelvis.

indomethacin in the two groups. The only difference found was related to the effect of (S)-ketoprofen ($10 \mu\text{M}$) on the maximal amplitude of depolarization which was more pronounced in group A ($29 \pm 8\%$ inhibition, $n = 4$) than in group B ($6 \pm 3\%$ inhibition, $n = 4$). Such a difference was not observed for indomethacin ($10 \mu\text{M}$) which produced a comparable inhibitory effect in the two groups (34 ± 8 and $36 \pm 6\%$ inhibition, $n = 4$ each).

A tenfold greater concentration of (S)-ketoprofen ($100 \mu\text{M}$, $n = 4$, 3 from group A) produced a time-dependent shortening of action potential duration (49% reduction), a reduced amplitude of contraction (46% inhibition) and a shortening of contraction duration (50% inhibition), not significantly different from the effect produced at $10 \mu\text{M}$. At $100 \mu\text{M}$ (S)-ketoprofen also produced a significant reduction of the maximal amplitude of depolarization ($23 \pm 6\%$ inhibition, $P < 0.05$).

When 100 μM indomethacin was added to the superfusion medium 60 min after superfusion with 100 μM (S)-ketoprofen, it produced a prompt suppression of the residual action potential and contraction (Figure 7b).

Evoked action potentials in the ureter

As described in previous studies (Maggi *et al.*, 1994a,b), the capsaicin-pretreated guinea-pig ureter in the presence of Bay

Table 1 Parameters of spontaneous (pacemaker) potentials and accompanying phasic contractions of the guinea-pig renal pelvis (sucrose gap)

	Group A (n = 22)	Group B (n = 23)
Action potential		
Action potential		
Frequency (cycles min^{-1})	2.01 ± 0.18	$1.25 \pm 0.08^*$
Amplitude of depolarization (mV)	7.85 ± 0.54	$11.95 \pm 0.47^*$
Time to peak (ms)	962 ± 81	$543 \pm 36^*$
Rate of depolarization (mV s^{-1})	8.9 ± 0.67	$24.1 \pm 1.94^*$
Duration (ms)	2683 ± 139	3156 ± 314
Repolarization (ms)	1722 ± 137	$2613 \pm 321^*$
After hyperpolarization (mV)	1.25 ± 0.22	1.55 ± 0.20
Mechanical activity		
Amplitude (mN)	4.70 ± 0.33	4.98 ± 0.39
Time to peak (ms)	1789 ± 88	1933 ± 140
Duration (ms)	3946 ± 142	4022 ± 348
Relaxation time (ms)	1707 ± 80	2088 ± 217

Each value is mean $\pm$ s.e.mean. The preparations were subgrouped to meet two criteria: (a) rate of depolarization $<$ or $>$ than 15 mV; (b) absence or presence of a plateau in the action potential. Nine out of 54 preparations studied failed to meet both criteria of inclusion: of the remainders, 22 were classified as group A and 23 as group B. *Significantly different from group A ($P < 0.05$).

K 8644 (1 μM) responds with regular action potentials and phasic contractions following repetitive cycles of direct electrical stimulation (1–2 ms pulse width, 10–20 V). (S)-ketoprofen and indomethacin (10 μM for 60 min, $n = 5$ for each drug) did not significantly affect the action potential and contraction of the guinea-pig ureter as compared to control preparations superfused for 60 min with drug-free Krebs solution ($n = 5$). The only effect produced by COX inhibitors was depolarization of the membrane: this effect ensued at 10–20 min and reached a steady state at 40–60 min from start of superfusion with (S)-ketoprofen or indomethacin. At 60 min from the start of superfusion, the effect of (S)-ketoprofen and indomethacin averaged 3.21 ± 0.78 and 2.03 ± 0.34 mV ($n = 5$), while no significant changes were observed in controls.

Discussion

Electromechanical coupling in the upper urinary tract

The smooth muscle of the upper urinary tract (renal pelvis and ureter) relies on electromechanical coupling to accomplish its physiological function of transport of urine. Action potentials are spontaneously generated by pacemaker cells in the renal pelvis while the ureter smooth muscle is normally quiescent unless invaded by action potentials travelling through the ureter to produce a peristaltic wave. Phasic contractions are invariably preceded by action potentials in both the renal pelvis and ureter and L-type calcium channel blockers suppress the generation of action potentials and contractility (Weiss 1992 for review; Shuba 1977; Brading *et al.*, 1983). This concept is further strengthened here by the fact that nifedipine was found to eliminate totally action potentials and contractility in the renal pelvis, evoked contractility of the ureter and propagation of impulses through the ureter.

Figure 6 Time-dependent changes in amplitude and duration (at 50% of repolarization) of the action potential and amplitude and duration of contraction (at 50% of relaxation) observed in preparations superfused for 60 min with drug-free Krebs solution (controls, $\circ$), 10 μM (S)-ketoprofen ($\square$) or 10 μM indomethacin ($\triangle$). Each value is mean $\pm$ s.e.mean of 11–13 experiments. *Significantly different from baseline values, $P < 0.05$.

Figure 7 (a) Effect of (S)-ketoprofen on pacemaker potential and contraction of the guinea-pig renal pelvis. (b) The (S)-ketoprofen-resistant pacemaker potentials of the guinea-pig renal pelvis are promptly suppressed by 100 μ M indomethacin. In both panels, the lower tracing shows changes in membrane potential and the upper tracing shows tension.

It is currently held that prostanoids play a local autocrine and paracrine role regulating excitation-contraction coupling in the upper urinary tract (see Introduction for references). Crucial for this interpretation is the notion that blockers of prostanoid generation inhibit or suppress excitation-contraction coupling at this level: several COX inhibitors have been used in experiments on the isolated renal pelvis and ureter smooth muscle from different species yet, as outlined in the Introduction, the exact importance of locally generated prostanoids in setting excitation-contraction coupling in the upper urinary tract is unclear, especially from a quantitative point of view. We have re-addressed this issue by investigating the action of the two enantiomers of ketoprofen, a well known COX inhibitor. We speculated that the qualitative and quantitative differences observed between the action of (S)- and (R)-ketoprofen would provide an exact estimate of the true importance of COX products in modulating excitation-contraction coupling at this level. A direct comparison with indomethacin also appeared of interest for re-interpretation of previous findings on this topic.

The estimate of the potency of COX inhibitors in blocking prostanoids production greatly varies with the assay employed: for instance, racemic ketoprofen is either less potent (about ten fold) or slightly more potent (about three fold) than indomethacin in inhibiting prostaglandin production from cultured macrophages and sheep seminal vesicle microsomes, respectively (Brune *et al.*, 1981); yet IC_{50} values in the low μ M range have been reported in various assays for inhibition of prostaglandin/thromboxane synthesis for both compounds (Rainsford, 1988 for review). Following the separation of the two enantiomers, COX inhibition by racemic ketoprofen was found to reside almost entirely in the (S)-enantiomer which, in different cellular models, is 33–3,000 fold more potent than the (R)-enantiomer (Suesa *et al.*, 1993). Indeed, contamination by small amounts of (S)-ketoprofen (about 0.5%) may well account for COX inhibition observed when using high concentrations of the (R)-enantiomer. The present findings demonstrate that, up to 10 μ M, the effects of (S)-ketoprofen and indomethacin on motility of the guinea-pig isolated renal pelvis are essentially

superimposable. At concentrations equal or greater than 100 μ M, indomethacin produces a general suppressant effect on spontaneous and evoked motility of the pyeloureteral system. These effects are not shared by (S)-ketoprofen and are likely to involve a nonspecific action of indomethacin (Northover, 1971; 1973; Whittle, 1976) on membrane channels, excitability and contractility.

Stereoselective COX inhibition and renal pelvis motility

The present findings indicate that endogenous prostanoids play a modulatory and amplifying role in spontaneous excitation-contraction coupling of the guinea-pig renal pelvis, yet also indicate that the generation of endogenous prostanoids is nonessential for this process: pacemaker potentials modified in their shape/duration were observed after 60 min superfusion with 10 or 100 μ M (S)-ketoprofen and each of them was able to produce contraction. Although the possibility that species-related differences exist in this respect, the total suppression of spontaneous contractions of the sheep and rabbit renal pelvis observed with 10–100 μ M indomethacin (Lundstam *et al.*, 1985; Thulesius *et al.*, 1986) is likely to arise from a mixed effect on COX and nonspecific effects on excitation-contraction coupling.

The interpretation of the physiological relevance of the present findings is somewhat complicated by the notion that mechanical trauma increases the production of prostanoids: therefore the basal prostanoids production (and consequently the intensity of the effects produced by inhibiting COX) may have been artificially increased by the dissection procedure. In this respect, it is worth noting that the frequency of spontaneous contractions of the isolated renal pelvis in organ bath experiments (4–7 cycles min^{-1}) is higher than the frequency of pacemaker potentials measured during continuous superfusion in sucrose gap (1–2 cycles min^{-1} in groups A and B).

At least two factors could be important in determining this difference. On the one hand, there is evidence that in species with unicalyceal kidney (e.g. guinea-pig and rabbit) specialized muscle cells with putative pacemaker function are distributed in the whole renal pelvis (Gosling & Dixon,

1971), yet the basic frequency of pacemaker potentials shows a distinct proximal-to-distal gradient. Each small region of the renal pelvis behaves as an oscillator and the integrated function is best described as a hierarchically organized multiple-coupled pacemaker system (Constantinou & Tamaguchi, 1981; Kimoto & Constantinou, 1991). In sucrose gap, the multicellular recording of electrical activity originates from a small region of the sample: it appears conceivable that, in preparations having the lower frequencies of pacemaker activity (group B) the area of recording was closer to the ureter. This is further suggested by the presence of a pronounced plateau in the action potential in group B, since this is a well characterized feature of the action potential of the guinea-pig ureter (Shuba, 1977; Brading *et al.*, 1983). By contrast recordings made from preparations in group A are likely to involve more proximal regions of the renal pelvis where pacemaker cells with higher intrinsic frequencies are located. On the other hand, in organ bath experiments, the activity of the whole renal pelvis is recorded, which is anyway driven by the pacemaker cell(s) with the highest intrinsic frequency of discharge.

A second factor may be linked to the continuous superfusion performed in sucrose-gap, enabling the removal of locally released mediators (such as prostanoids). We observed that the renewal of bathing solution by washout slows down the frequency of contractions of the renal pelvis in organ bath experiments, and some minutes are needed for recovery to the original values of frequency and amplitude. Thus, endogenous prostanoids may accumulate in the organ bath fluid to provide an optimal reinforcement of the activity of pacemaker cells. Either one of these factors may explain the higher values of spontaneous activity observed in organ bath vs. sucrose gap experiments.

The mean values of frequencies (and other parameters) were not significantly different from each other in the experimental groups aimed to analyse the effect of (S)-ketoprofen and indomethacin (10 μ M each) vs. spontaneous time-related changes in sucrose gap experiments. This reflects a balanced distribution of preparations from groups A and B in the three experimental groups (controls, (S)-ketoprofen and indomethacin). When the effects of (S)-ketoprofen and indomethacin were analysed for possible differential effects in groups A and B, the only difference was found about the inhibitory effect of (S)-ketoprofen on the amplitude of depolarization which, at 10 μ M, was apparently larger in group A than in group B. Overall, the present findings do not support the possibility of a differential effect of prostanoids on excitation-contraction coupling in different regions of the guinea-pig renal pelvis, yet the limitation of the technique (multicellular recording) leaves this point open to further investigation. Considering the effect of 10 μ M (S)-ketoprofen as a pure consequence of full COX inhibition, it would follow that modulation of action potential duration is the main mechanism through which endogenous prostanoids regulate pacemaker potentials of the guinea-pig renal pelvis. These findings suggest an amplifying or facilitatory effect of endogenous prostanoids in L-type calcium current which sustains the pacemaker of the renal pelvis.

Stereoselective COX inhibition and ureter motility

Previous studies have suggested a facilitatory influence of endogenous prostanoids on excitation-contraction coupling

of the human and ovine isolated ureter. Contrary to the guinea-pig, the isolated ureter of the sheep is spontaneously active, although the frequency of spontaneous contraction is irregular and anyway lower than in the renal pelvis (Thulesius & Angelo-Khattar, 1985; Thulesius *et al.*, 1986). Indomethacin (10 nM–100 μ M) was shown to produce a complete blockade of this spontaneous activity, the time required for full blockade being inversely proportional to the concentration tested (Thulesius & Angelo-Khattar, 1985). The human isolated ureter was reported to be either spontaneously active and blocked by indomethacin (Angelo-Khattar *et al.*, 1985) or quiescent (Cole *et al.*, 1988); in the latter study, 100 μ M indomethacin blocked contractions evoked by direct stimulation of smooth muscle. Prostaglandins of the E and F series were found to be excitatory in both species (Thulesius & Angelo-Khattar, 1985; Cole *et al.*, 1988).

The guinea-pig ureter is electrically and mechanically quiescent: prostaglandins of the F series are inactive while prostaglandins of the E series produce muscle relaxation and membrane hyperpolarization, both effects being possibly linked to adenylate cyclase stimulation and elevation of cyclic AMP (Johns & Wooster, 1975; Vermue & Den Hertog, 1987). It appears conceivable that the slowly developing and sustained depolarization of the ureter observed in this study is ascribable to the removal of endogenous prostanoids by COX inhibition. However, this modulatory influence had no apparent major influence on the action potential and contractility of the ureter nor on the propagation of impulses along the ureter. High concentrations of indomethacin suppressed the evoked motility of the guinea-pig ureter: the involvement of COX inhibition appears highly debatable since (S)-ketoprofen was ineffective when tested at similarly high concentrations.

In all, our findings do not lend support to the idea that endogenous prostanoids play a major role in regulating electromechanical coupling and propagation of impulses along the guinea-pig ureter. Previous studies have suggested that the pyeloureteral junction may be a crucial target for action of endogenous prostanoids in regulating propagation of impulses to the ureter (Kimoto & Constantinou, 1991). Our data do not allow speculation on this matter because of the technical problem in isolating and studying such a small region of the guinea-pig pyeloureteral system.

Pathophysiological implications

The reinforcement of pacemaker activity of the renal pelvis by endogenous prostanoids demonstrated here could be of importance for the effect of COX inhibitors on renal colic. In addition to a possible central analgesic effect, a reduction of renal blood flow and urine production is held as a major mechanism through which COX inhibitors produce pain relief in renal colic (e.g. Perlmutter *et al.*, 1993). Assuming that endogenous prostanoids reinforce spontaneous excitation-contraction coupling in the renal pelvis, blockade of this effect by COX inhibitors may contribute to the overall pain relief produced by these drugs in renal colic, by reducing intraureteral pressure.

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(Received July 26, 1994
Revised November 14, 1994
Accepted November 16, 1994)