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Effects of selective inhibitors of cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) on the spontaneous myogenic contractions in the upper urinary tract of the guinea-pig and rat

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1 The role of cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) in the upper urinary tract of the guinea-pig and rat was examined using simultaneous tension recordings of the proximal and distal regions of the renal pelvis and the ureter.

2 The guinea-pig upper urinary tract contracted at a frequency (7.52 ± 0.3 min^{-1} at 35°C) significantly lower than the frequency in the proximal renal pelvis (21.6 ± 1.3 min^{-1}) and in the distal renal pelvis and ureter (20.2 ± 1.4 min^{-1}) of the rat (at 30°C).

3 Indomethacin ($\geqslant 1$ μM for 60 min), decreased the motility index (amplitude × frequency) (MI) in all three regions of the guinea-pig upper urinary tract, an effect which mainly arose from a decrease in the frequency of contractions. In the rat, indomethacin (1–30 μM for 60 min) significantly decreased the MI calculated in the proximal renal pelvis ($\geqslant 30$ μM indomethacin), and in the distal renal pelvis ($\geqslant 10$ μM indomethacin), arising from a significant decrease in the amplitude of contractions.

4 The COX-1 inhibitor, valeryl salicylate (VSA) (5–100 μM for 60 min), had no effect on either the amplitude or frequency of contractions in the guinea-pig upper urinary tract. In contrast, VSA increased the force of contractions in the proximal and distal renal pelvis of the rat, whilst having little effect on the frequency of contractions.

5 The COX-2 inhibitor, NS-398 (10–100 nM for 60 min) reduced the MI in the guinea-pig upper urinary tract in a concentration-dependent manner. The MIs calculated for the proximal renal pelvis, distal renal pelvis and ureter, were decreased by 72, 64 and 72% respectively, in 100 nM NS-398. NS-398 (10–100 nM) had no effect on any of the three parameters measured in either the proximal or distal renal pelvis of the rat.

6 These data suggest that endogenously-released prostaglandins (PGs) maintain the myogenic contractility of the upper urinary tract in both the guinea-pig and rat. Moreover COX-2 is the primary enzyme involved in synthesizing PGs in the guinea-pig upper urinary tract, while COX-1 appears to be the predominantly active enzyme in the rat.
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Abbreviations: ANOVA, analysis of variance; COX-1, cyclo-oxygenase-1; COX-2, cyclo-oxygenase-2; DRP, distal renal pelvis; MI, motility index; NS-398, N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulphonamide; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PGH_2, prostaglandin H_2; PGHS, prostaglandin endoperoxide synthase; PRP, proximal renal pelvis; SC-19220, 1-acetyl-2-[8-chloro-10,11-dihydrodibenz (b,f)(1,4) oxazepine-10-carbonyl] hydrazine; VSA, valeryl salicylate

Introduction

Prostaglandins (PGs) are found throughout the body and function as local hormones playing a role in many different biological processes such as haemostasis, modulation of kidney and gastric function, inflammation and maintenance of smooth muscle contractility. PGs are formed upon conversion of arachidonate to prostaglandin H_2 (PGH_2), which is then further synthesized into the many different forms of prostaglandins (such as PGE_2, PGI_2, $PGF_{2\alpha}$). The key enzyme in allowing this conversion to take place is prostaglandin endoperoxide synthase (PGHS) (Herschman, 1996), which through its cyclo-oxygenase activities converts free arachidonic acid to prostaglandin G_2 (PGG_2), which is then further converted to PGH_2 by the hydroperoxidase activity of PGHS. Recently, two isoforms of the PGHS enzyme (also referred to as cyclo-oxygenase) have been discovered, cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2). COX-1 is constitutively expressed in most tissues and in general, produces PGs involved in regulating normal 'housekeeping' cellular processes (DeWitt *et al*., 1993; Bhattacharyya *et al*., 1995). In contrast, COX-2 is undetectable in most tissues under normal physiological conditions. However, it can be rapidly and transiently induced (by as much as 10–80 fold) during inflammation or in cultured cells after exposure to mitogenic stimuli (Meade *et al*., 1993; Smith *et al*., 1994).

Over the past couple of decades, non-steroidal anti-inflammatory drugs (NSAIDs) have been used to block PGHS to reduce inflammation as well as act as analgesic agents. However, NSAID-induced side effects have been demonstrated in the gastrointestinal tract and kidney, where the disruption of the production of PGs reduces acid production and cytoprotective mucus formation, which in turn leads to dyspepsia and the formation of ulcers (DeWitt *et al*., 1993). It is already well established that NSAIDs, such as

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indomethacin and aspirin, inhibit both COX-1 and COX-2 (Meade *et al*., 1993; Bhattacharyya *et al*., 1995; Charette *et al*., 1995). Recently, a number of inhibitors relatively selective for COX-1 or COX-2 have been discovered, each of them demonstrating differing degrees of potency in their ability to inhibit cyclo-oxygenase activity. Two such drugs are valeryl salicylate (VSA) and NS-398. VSA has been shown to inhibit production of PGs (by 85–90%), in populations of cos-1 cells transfected with cDNAs encoding human COX-1, whilst having little effect on COX-2 (Bhattacharyya *et al*., 1995). On the other hand, NS-398 has been demonstrated to selectively inhibit COX-2 activity by causing an irreversible structural transformation of the enzyme (Copeland *et al*., 1994; Futaki *et al*., 1994), having the same potency as indomethacin in inhibiting PG production in rats which have carrageenin-induced paw edema, but having no effect in non-inflamed tissue. NS-398 has also been demonstrated to be less toxic compared to standard NSAIDs, inducing little gastric damage in rats (Futaki *et al*., 1993; 1994).

The development of relatively selective COX inhibitors has also allowed investigations into normal physiological processes, which require the endogenous synthesis and release of PGs. The upper urinary tract of many mammalian species displays spontaneous peristaltic contractions which are generally thought to be myogenic in origin, being little affected by tetrodotoxin or by blockers of autonomic nerve function (Golenhofen & Hannappel, 1973; Thulesius & Angelo-Khattar, 1985; Maggi & Giuliani, 1992; Maggi *et al*., 1992). However, the application of a PG antagonist (SC-19920) or non-selective COX inhibitors (indomethacin and acetylsalicylic acid) decreased the amplitude and sometimes the frequency of spontaneous contractions occurring in muscle strips of the rabbit, sheep and guinea-pig renal pelvis, which was reversed upon addition of $PGF_{2\alpha}$ or dinoprost, a stable $PGF_{2\alpha}$ analogue (Thulesius & Angelo-Khattar, 1985; Thulesius *et al*., 1987; Kimoto & Constantinou, 1991; Zhang & Lang, 1994; Santicioli *et al*., 1995). Indomethacin has also been shown to cause a concentration- and time-dependent decrease in the frequency and duration of the action potentials associated with the spontaneous contractions of the guinea-pig upper urinary tract. The rate of action potential discharge was, however, partially restored by dinoprost, suggesting that the continual synthesis and release of PGs is essential in the maintenance of these peristaltic contractions (Zhang & Lang, 1994; Santicioli *et al*., 1995). To date, there have been no investigations into the nature of the COX isoform involved in producing the PGs needed for the maintenance of these spontaneous contractions. In this report, we have compared the effects of NS-398 and VSA on the myogenic contractility in the upper urinary tract of both the guinea-pig and rat.

Methods

Guinea-pigs of either sex (200–400 g) were stunned and exsanguinated, while Wistar rats (200–500 g) were anaesthetized with chloroform followed by decapitation. Kidneys were removed through a midline incision and immediately placed in physiological saline (see below). The upper urinary tract was dissected free from the surrounding parenchyma and a longitudinal cut was made extending from the proximal renal pelvis through to the mid region of the ureter enabling the whole preparation to be laid out flat. Three circumferentially directed cuts, which extended to the midline were then made. The first cut divided the proximal portion of the renal pelvis from the distal renal pelvis, while two smaller cuts were made in the ureter (approximately 1 cm from the pelviureteric junction) to create a short circumferential strip. Threads were then tied around the three separated regions and the preparation was pinned into an organ bath (1.3 ml) perfused with physiological saline and maintained at a temperature of 35°C for guinea-pigs and 30°C for rats. The threads were attached to isometric force transducers, which were connected to a MacLab/4s, analogue-to-digital converter, driven by a Macintosh LC, thus allowing the simultaneous recording of tension developed in the three different regions of the upper urinary tract. A tension of approximately 1 mN was placed on each of the regions and the tissue was then left to equilibrate for a period of 30–60 min, after which time all three regions generated contractions which were regular in both amplitude and frequency (Teele & Lang, 1998).

After equilibration, the effects of three different COX inhibitors were examined. Valeryl salicylate (VSA) (5–100 μM), NS-398 (10–100 nM) and indomethacin (1–30 μM), were added separately to the bathing saline. Any effects of these COX inhibitors on the amplitude and frequency of the spontaneous contractions were measured after 60 min. Control experiments with no added inhibitors were also carried out over a time period of 240 min. This was done in order to investigate the effects of muscle fatigue over time that could possibly interfere with the interpretation of the results obtained after application of the COX inhibitors. Changes in contractility were measured every 30 min.

In general, the effects of treatment on the spontaneous contractility of the upper urinary tract were quantified by the use of a motility index (MI), calculated by multiplying the averaged amplitude (in mN) of five contractions by their frequency, expressed as the number of contractions min^{-1}. Amplitudes, frequencies and MIs obtained 60 min after perfusion of the tissue with a particular COX inhibitor, were then expressed as a per cent of their control values. Similarly in the control experiments, the amplitude and frequency of five contractions were averaged after regular contractions were established, and used as the control values at time zero. These values were then used to calculate the relative values (per cent of control) of these three parameters after 60, 120 and 180 min. Cumulative concentration-response curves were constructed by plotting data as a per cent of control against the concentration of the COX inhibitor. Time-matched control curves were also plotted on the same graph at the corresponding time points. In all experiments, control contractions to high K^+ (20 mM) saline (for 2 min) and/or dinoprost (2 μM for 1–2 min) were elicited before and during the application of a COX inhibitor to exclude the possibility that these COX inhibitors were having a non-specific blocking action.

Statistical analysis

Values given in figures are expressed as means ± standard error of the mean. A number of different statistical analyses were carried out to test for significance. A one-way repeated measures analysis of variance (ANOVA) was conducted where regions of the upper urinary tract were the repeated measures, and animal was a between subject factor. A two-way repeated measures ANOVA was also employed using region and time/concentration as within subject factors, and on some occasions incorporating drug as a between subject factor. Data was tested for homogeneity of variance, and transformed if necessary. Where significant interactions occurred, a Fisher's least significant difference test was then conducted to determine

where the differences occurred; $P<0.05$ was considered statistically significant.

Drugs

The physiological saline was of the following composition (in mM): NaCl 120, KCl 5.0, $CaCl_2$ 2.5, $MgCl_2$ 1.0, NaH_2PO_4 1.0, $NaHCO_3$ 25 and glucose 11. The pH of this solution was 7.3–7.4, after being bubbled with an O_2: CO_2 (95% O_2: 5% CO_2) gas mixture. The following drugs were used: valeryl salicylate (VSA) (Sapphire Bioscience), NS-398 (N-[2-cyclohexyloxy-4-nitrophenyl] methanesulfonamide) (Sapphire Bioscience) and indomethacin (Sigma). VSA was dissolved in dimethyl sulphoxide, NS-398 in ethanol, and indomethacin in 0.1 M Na_2CO_3. Drugs were diluted with physiological saline to their final concentrations as indicated. Before use, solutions were vigorously bubbled with 95% O_2: 5% CO_2 to restore any changes of pH.

Results

Spontaneous activity

The upper urinary tracts of both the guinea-pig and rat developed spontaneous contractions immediately upon being set up. After 30–60 min equilibration, the spontaneous contractions occurring in the guinea-pig upper urinary tract (at 35°C) were regular in both amplitude and frequency, contracting at a force of 4.31 ± 0.62, 2.97 ± 0.35 and 0.56 ± 0.09 mN ($n=10$) in the proximal and distal renal pelvis and ureter, respectively (Figure 1Ai–iii,Cai). All regions contracted at a common rate of 7.52 ± 0.3 min^{-1} (Figure 1Ai–iii,Caii, $n=10$). In contrast, the contractile activity recorded in the rat upper urinary tract (at 30°C) was more irregular in shape. The proximal and distal renal pelvis and the ureter of the rat developed contractions of 0.50 ± 0.08, 0.42 ± 0.08, and 0.18 ± 0.03 mN ($n=10$), respectively (Figure 1Bi–iii,Cbi), significantly lower than those developed in the corresponding regions of the guinea-pig upper urinary tract (Figure 1Cai, $P<0.05$). However the frequency of contractions in the rat was significantly greater, contracting at a rate of 21.6 ± 1.3 min^{-1} in the proximal renal pelvis, and 20.2 ± 1.4 min^{-1} in the distal renal pelvis and ureter (Figure 1Bi–iii,Caii), almost three times the frequency developed in the guinea-pig upper urinary tract (Figure 1Cbii). Cyclical movements of the baseline were often also apparent in the rat upper urinary tract (Figure 1B), compared with the constant baseline of the guinea-pig (Figure 1A). The MIs of the proximal and distal renal pelvis of the guinea-pig were also significantly greater than all regions of the rat, as well as the guinea-pig ureter (Figure 1Caiii,biii, $P<0.05$; 1-way repeated measures ANOVA).

The spontaneous contractions occurring in the guinea-pig upper urinary tract are generally thought to be myogenic in origin, as application of guanethedine, atropine or tetrodotoxin, have no effect on contractile amplitude or frequency (Golenhofen & Hannappel, 1973; Maggi *et al*., 1992; Teele & Lang, 1998). However these contractions are thought to be maintained by the tonic release of PGs and sensory neuropeptides (Golenhofen & Hannappel, 1973; Maggi & Giuliani, 1992; Maggi *et al*., 1992; Teele & Lang, 1998). Equivalent investigations have not been carried out in the rat upper urinary tract. The application of capsaicin (10 μM for 15 min, followed by 60 min washout) to the upper urinary tract of the rat, had no effect on either the amplitude or frequency of contractions, and hence the MI. Calculated MIs for the proximal and distal renal pelvis and ureter of the rat were 5.0 ± 1.8, 5.4 ± 1.2 and 2.2 ± 0.8 mN, respectively, compared to their control values of 5.4 ± 1.1, 6.3 ± 1.2 and 2.7 ± 0.8 mN ($P>0.05$, $n=8$, data not shown). Similarly, tetrodotoxin (3 μM) had no effect on the contractility in the upper urinary tract of the rat. After approximately 20 min exposure to tetrodotoxin (3 μM), MIs calculated in the proximal renal pelvis, distal renal pelvis and ureter (3 μM) decreased to 88, 87 and 79% respectively, of control values ($P>0.05$, $n=4$, data not shown). These results suggest that in contrast to the guinea-pig, the spontaneous contractions occurring in the upper urinary tract of the rat are not maintained through the tonic release of neuropeptides from capsaicin-sensitive nerves.

Five control experiments were carried out in both species to investigate any effects of muscle fatigue over a time period of 240 min. The amplitude of contractions recorded in all regions of the guinea-pig upper urinary tract slowly decreased, with all time points ($\geqslant$15 min) being significantly lower than the control value (Figure 2Ai). No significant difference between regions was evident. In contrast, the force of contractions in the rat renal pelvis increased over time in the proximal and distal renal pelvis, with time points$\geqslant$120 min being significantly greater than control. At 180 min, the force of contractions in the proximal and distal renal pelvis were increased by 33 and 17%, respectively (both $P<0.05$). There was no time-dependent change in contraction amplitudes in the rat ureter (Figure 2Bi). In both the guinea-pig and rat upper urinary tract, the frequency of contractions in all three regions progressively declined over time, with values reaching significance at time points$\geqslant$120 min (Figure 2Aii, Bii). Relative MIs calculated for the guinea-pig were found to be significantly lower at all time points measured in all three regions (Figure 2Aiii), reflecting the decrease seen in both the amplitude and frequency of contractions. In contrast, MIs calculated for the rat, did not change with time in the proximal and distal renal pelvis. In the rat ureter, MI values were significantly reduced at$\geqslant$60 min (Figure 2Biii, $P<0.05$, 2-way repeated measures ANOVA).

Effects of VSA

The COX-1 inhibitor, VSA was sequentially administered to the bathing saline at three concentrations, 5, 50 and 100 μM (each for a time period of 60 min) and cumulative concentration-dependent curves were constructed for both the guinea-pig and the rat upper urinary tract. Contraction amplitudes, frequencies and MIs were expressed as a per cent of control and plotted against time-matched control data recorded at 60, 120 and 180 min, corresponding to the time points when the effects of a particular VSA concentration was measured.

Although VSA superficially appeared to produce a concentration-dependent reduction in all three parameters and in all regions of the guinea-pig upper urinary tract ($n=5$), this change was not significant as the control data also decreased in a time-dependent manner. Thus, VSA had no significant effect on the MI values calculated after addition of the three concentrations of VSA, when compared to control (Figure 3A, $P>0.05$, 2-way repeated measures ANOVA).

In contrast, addition of VSA (5–100 μM) increased the amplitude of contractions in both the proximal and distal renal pelvis of the rat. This change in force, however, was only significantly different in the distal renal pelvis at concentrations$\leqslant$50 μM, where amplitudes were increased to 232 ± 11% (time-matched control 96 ± 9%, $n=5$) and 237 ± 18% (control 130 ± 18%) in 5 μM and 50 μM VSA, respectively ($P<0.05$, $n=7$, data not shown). VSA also appeared to cause a small

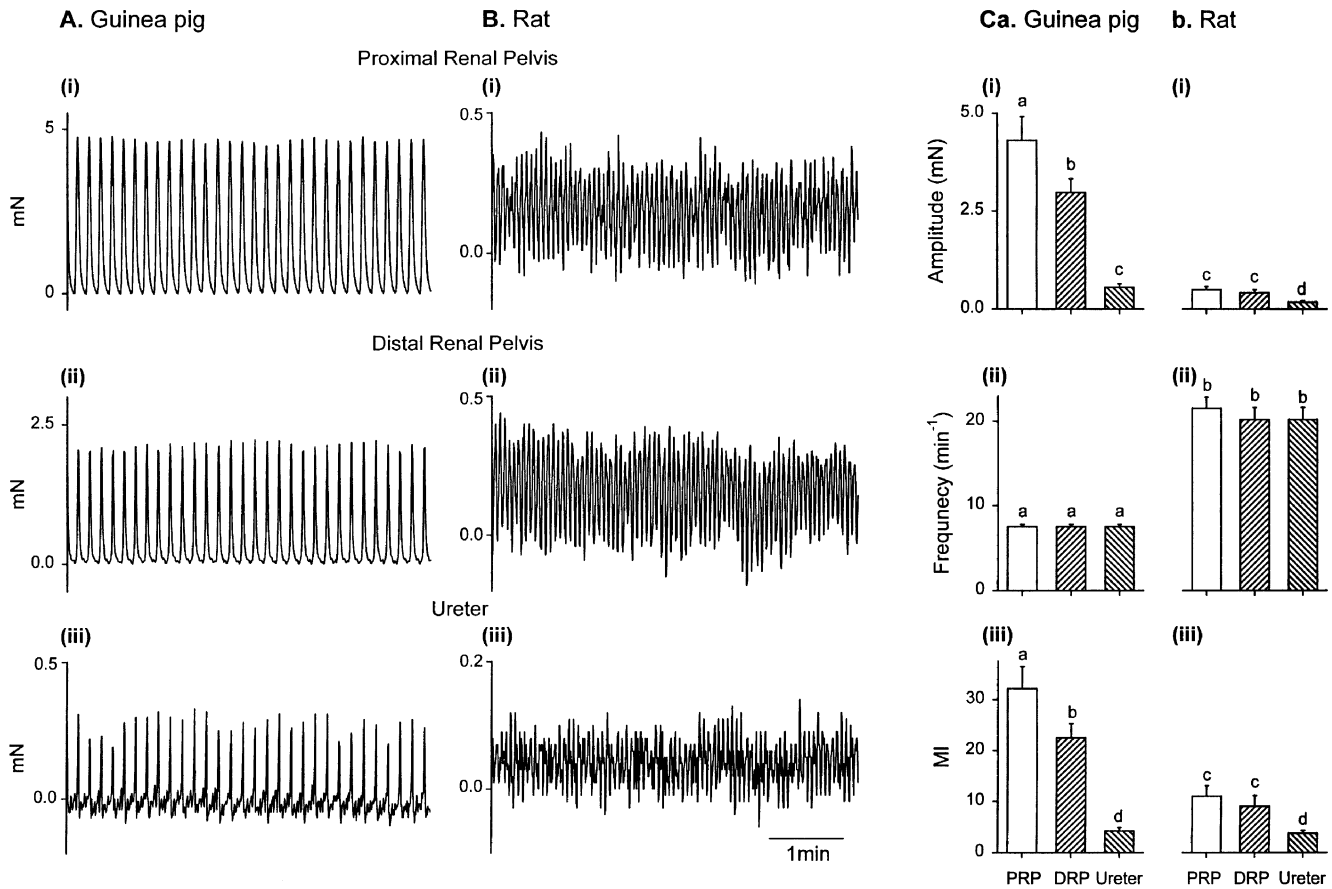

Figure 1 Typical example of the spontaneous contractions in the proximal renal pelvis (PRP) (Ai,Bi), and distal renal pelvis (DRP) (Aii,Bii), and ureter (Aiii,Biii) recorded simultaneously in either the guinea-pig (A) or rat (B). The averaged amplitude (Cai,bi) and frequency (Caii,bii) of contractions in all three regions were measured and the motility index (MI) (amplitude × frequency) (Caiii,biii) calculated ($n=10$). Mean values which do not share the same superscript are significantly different to one another ($P<0.05$; 1-way repeated measures ANOVA).

decrease in the frequency of contractions in both regions of the rat renal pelvis, which on the surface appeared to be dose-dependent. Statistical analysis, however, revealed that there was no significant difference between the frequency measured in the three concentrations of VSA (5–100 μM), and the time-matched control frequencies ($P<0.05$, data not shown). In Figure 3B, the MIs calculated for VSA (5–100 μM) have been compared against the time-matched control MIs. In 5 μM VSA, the calculated MIs in both regions of the renal pelvis were significantly greater than control, while at 100 μM VSA, the calculated MIs were significantly smaller ($P<0.05$). At 50 μM VSA, there was no difference in MI values compared to control (Figure 3B, $n=7$, $P>0.05$, 2-way repeated measures ANOVA). Data from the ureter of the rat has not been presented, due to the ureter being quiescent in three out of the seven experiments. In the remaining four experiments, the MI values decreased to 91, 74 and 29% of control values after 5, 50 and 100 μM VSA respectively.

Effects of NS-398

Addition of the COX-2 inhibitor, NS-398 (10–100 nM) to the guinea-pig upper urinary tract caused a concentration-dependent decrease in the calculated MI. In the proximal and distal renal pelvis, and the ureter, 60 min after perfusion with 100 nM NS-398, relative MIs were decreased to 28, 36 and 28% respectively, of their time-matched controls (Figure 4A). This reduction arose mainly from a significant decrease in the frequency of contractions recorded in all three regions of the upper urinary tract. After 60 min exposure to 100 nM NS-398, the frequency of contractions was reduced to $31\pm6\%$ compared to their time-matched control frequency of $73\pm8\%$ ($P<0.05$, $n=5$, data not shown). However, NS-398 (10–100 nM) caused no significant changes in the amplitude and frequency of contractions recorded in the proximal and distal renal pelvis of the rat, compared with the time-matched controls, and hence had no affect on the relative MIs (Figure 4B, $P>0.05$ $n=5$, 2-way repeated measures ANOVA). In the ureter of the rat, MI values calculated after 10 nM, 30 nM and 100 nM NS-398 decreased to 65, 52 and 67% of control values, respectively. However, this data was obtained only from two experiments, the other three preparations showing no spontaneous activity in the ureter (data not shown).

Effects of indomethacin

The effects of the non-specific COX inhibitor, indomethacin, have previously been examined in circumferentially-cut strips of tissue of the guinea-pig renal pelvis. Indomethacin was demonstrated to cause a concentration-dependent decrease in the amplitude and frequency of the spontaneous contractions in both the proximal and mid renal pelvis, causing complete cessation of contractions at concentrations $\geqslant 30$ μM (Zhang & Lang, 1994). In the present study, indomethacin caused a concentration-dependent decrease of the frequency of contractions, which was significantly different from control values at

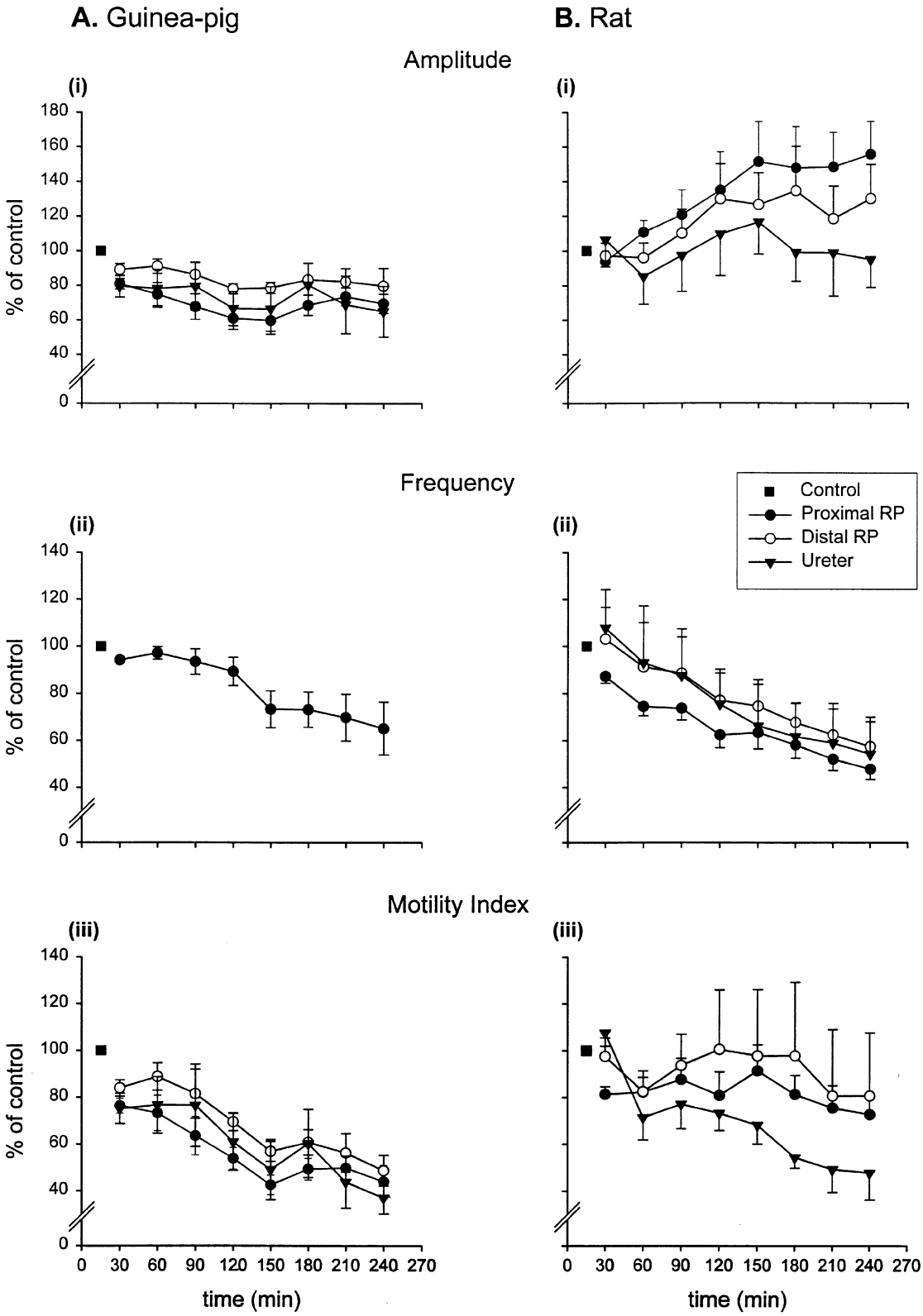

Figure 2 The effects of time on the amplitude (Ai,Bi) and frequency (Aii,Bii) of contractions and the calculated MIs (Aiii,Biii) in the proximal and distal renal pelvis, and the ureter of the guinea-pig (A) and rat (B).

indomethacin concentrations $\geqslant 10$ μM (data not shown). The effects of indomethacin on the amplitude of contractions were variable. For example, the amplitude of contractions in the proximal renal pelvis was significantly decreased at all indomethacin concentrations. In the distal renal pelvis, indomethacin had little effect on contractile amplitude, while in the ureter there was a significant increase in contractile force at indomethacin concentrations $\geqslant 10$ μM (data not shown). However, despite these variable results, the MIs calculated for both the proximal and distal renal pelvis were significantly reduced at all three concentrations of indomethacin (Figure 5A). The final MI values calculated after 60 min perfusion with

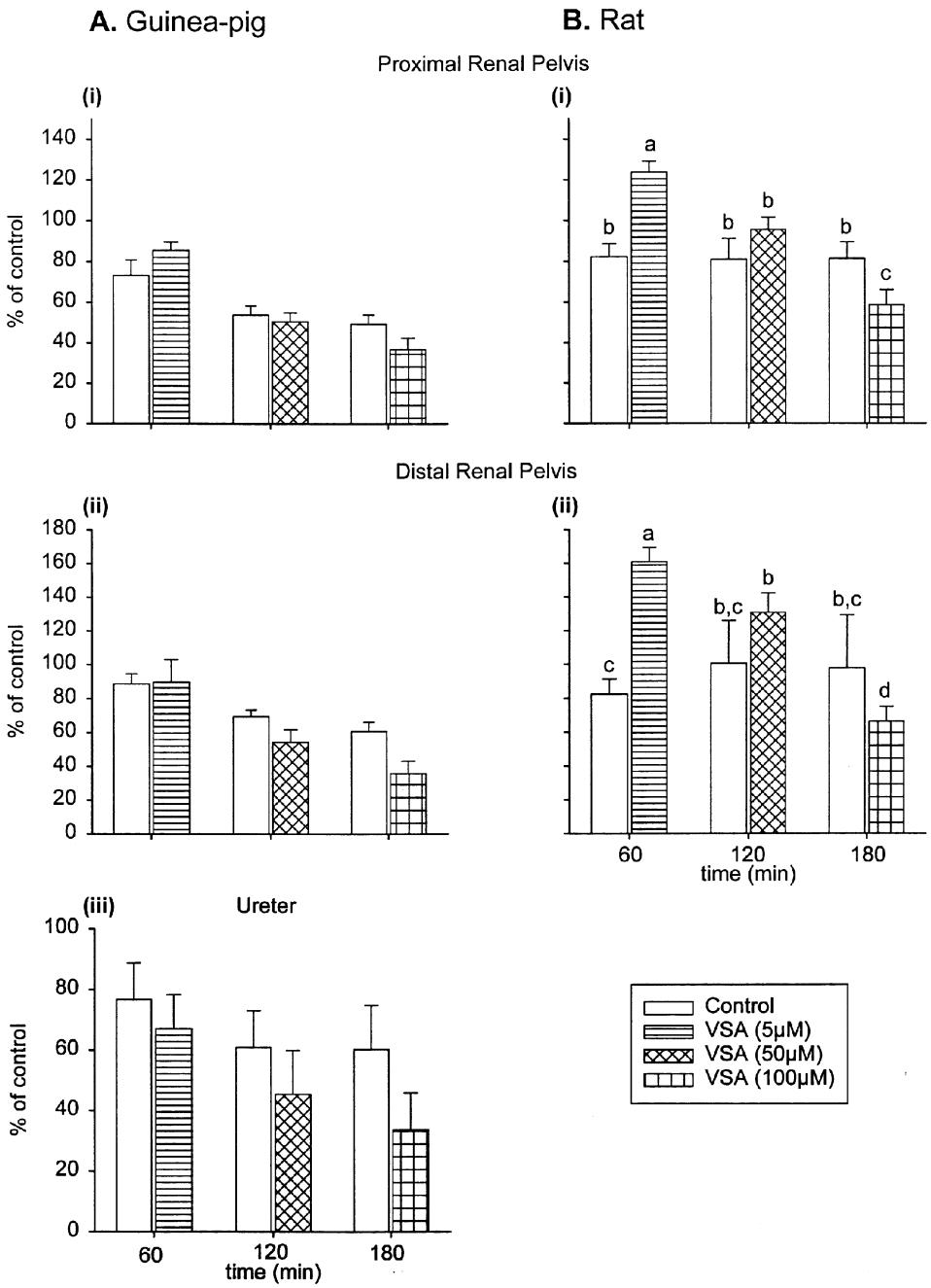

Figure 3 Pooled data on the effects of VSA (5–100 μM) on the calculated MIs in the upper urinary tract of the guinea-pig (A, $n=5$) and rat (B, $n=7$) compared to the time-matched control MIs ($n=5$). Mean values which do not share the same superscript are significantly different to one another ($P<0.05$; 2-way repeated measures ANOVA). Absence of letters denotes no significant difference.

30 μM indomethacin decreased to 22% ($P<0.05$), 47% ($P<0.05$) and 73% ($P>0.05$) of the time-matched controls in the proximal, distal renal pelvis and ureter, respectively (Figure 5A, $n=5$, 2-way repeated measures ANOVA).

In the rat, the MI values calculated for the proximal and distal renal pelvis were also decreased by indomethacin in a concentration-dependent manner (1–30 μM). However, in the proximal renal pelvis, this decrease was only significantly different from the time-matched controls with 30 μM indomethacin (Figure 5Bi). In contrast, the MIs calculated in the distal renal pelvis were significantly reduced at indomethacin concentrations $\geqslant 10$ μM (Figure 5Bii). MI values calculated in the rat ureter, also decreased in a concentration-dependent manner, decreasing to 87, 58 and 31% of control values after application of 1, 10 and 30 μM indomethacin respectively ($n=4$, data not shown). In comparison to the guinea-pig upper urinary tract, this reduction of the MI in the rat arose mainly through a decrease in the amplitude of the spontaneous contractions, with all three concentrations of indomethacin (1–30 μM) significantly reducing the contraction amplitudes recorded in both the proximal and distal renal pelvis ($P<0.05$), when compared with time-matched controls (data not presented). The frequency of contractions in the rat upper urinary tract was little affected by the non-specific COX inhibitor, indomethacin (1–30 μM) ($P>0.05$, $n=5$, 2-way repeated measures ANOVA, data not shown).

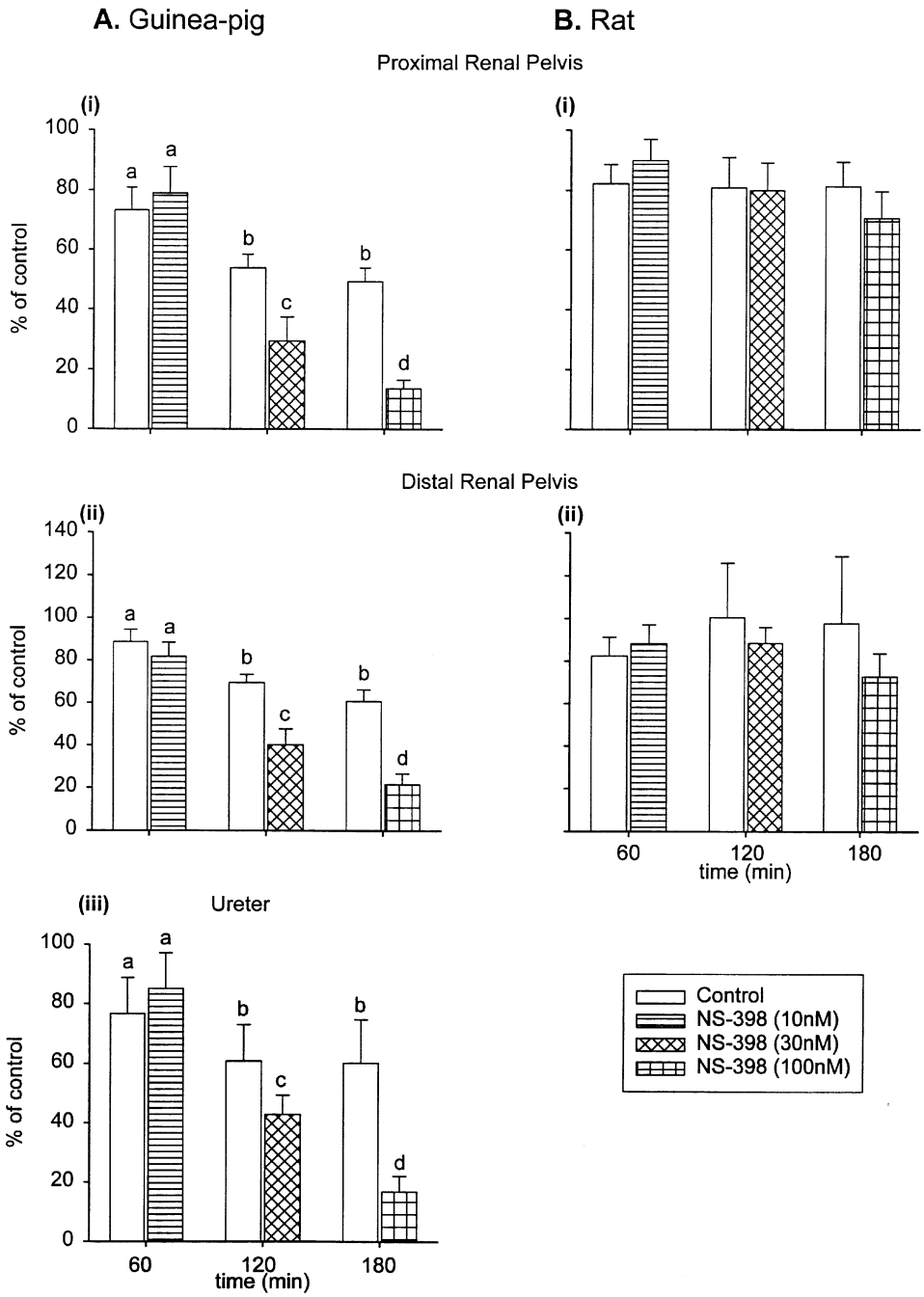

Figure 4 The effects of NS-398 (10–100 nM) on calculated MIs in the proximal and distal renal pelvis of the guinea-pig (Ai,ii) and rat (Bi,ii) and also the ureter of the guinea-pig (Aiii, $n=5$). Data is compared to time-matched controls. Mean values which do not share the same superscript are significantly different to one another ($P<0.05$; 2-way repeated measures ANOVA).

Discussion

Indomethacin has long been thought to be a non-selective inhibitor of the COX enzyme being equipotent at inhibiting COX-1 ($IC_{50}=7.4\times10^{-7}$ M) and COX-2 ($IC_{50}=9.7\times10^{-7}$ M) (Futaki *et al*., 1994). However other studies have indicated that indomethacin may well preferentially inhibit COX-1 over COX-2. For example, in mouse cos-1 cells transfected with either COX-1 or COX-2, indomethacin appeared to be approximately 20 fold better at inhibiting COX-1 than COX-2 (DeWitt *et al*., 1993; Smith *et al*., 1994). This uncertainty has led to the search for other agents that selectively inhibit COX-1 or COX-2. VSA (500 μM) has been shown to cause 85–90% inhibition of the PG production by COX-1, while inhibiting COX-2 activity by only 15%, thus exhibiting a 4–5 fold selectivity towards COX-1 (Bhattacharyya *et al*., 1995). NS-398, on the other hand, is a highly specific COX-2 inhibitor, showing more than 300 fold selective inhibition of COX-2 over COX-1 (Kargman *et al*., 1996). Biochemically, NS-398 has also been demonstrated to inhibit COX-2 enzyme (isolated from sheep placenta) with an IC_{50} value of 3.8×10^{-8} M, while COX-1 activity (isolated from ram seminal vesicles), was unchanged even at NS-398 concentrations of 10^{-4} M (Futaki *et al*., 1994). Even though it appears likely that NS-398 is a selective inhibitor of COX-2, a wide variation in its sensitivity has been reported, which might be attributed to the different experimental and assay conditions used (Griswold & Adams, 1996).

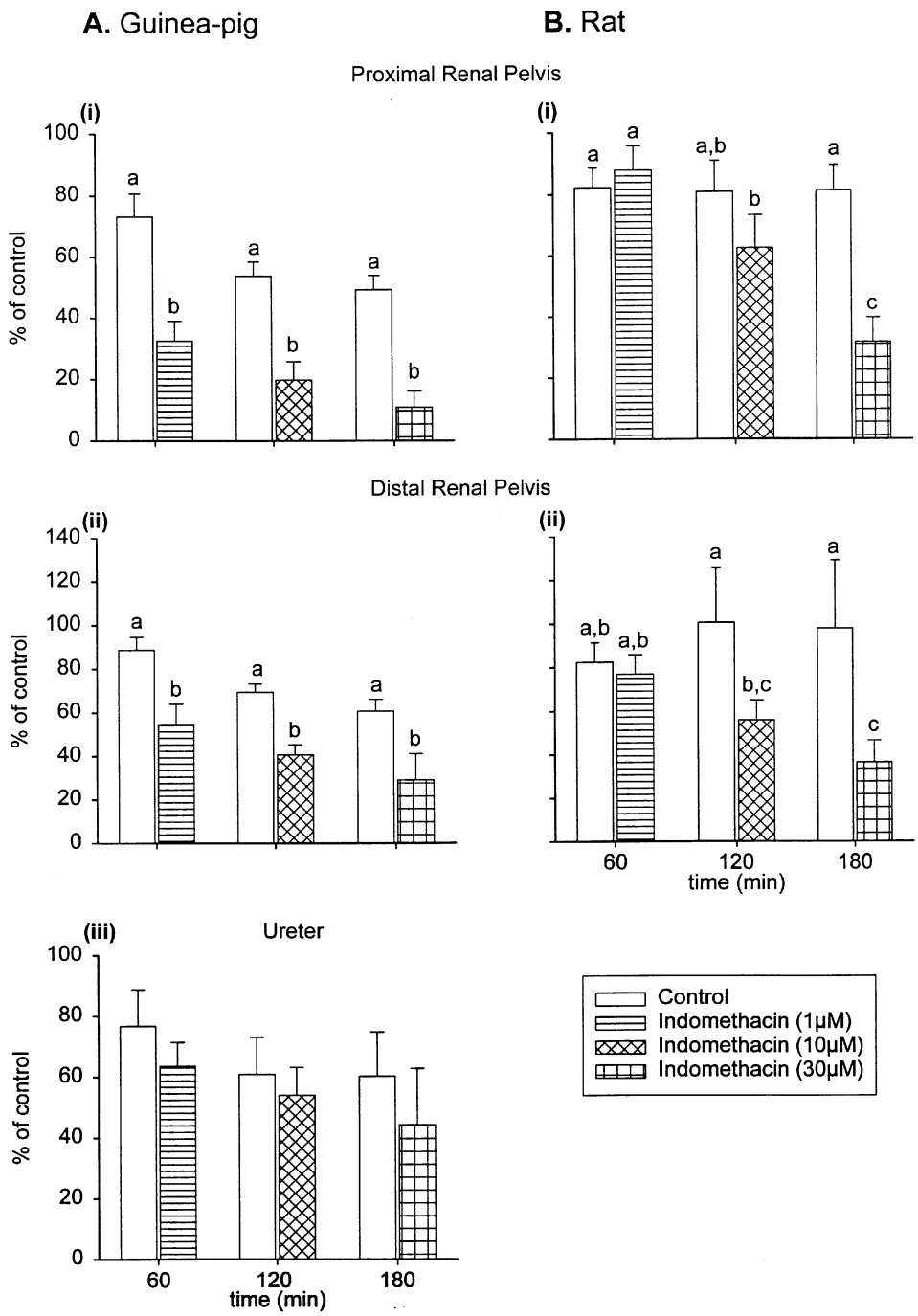

Figure 5 The effects of indomethacin (1–30 μM, applied for 60 min) on the MIs calculated in the guinea-pig (Ai–iii, $n=5$) and rat (Bi–ii, $n=5$) upper urinary tract. Mean values which do not share the same superscript are significantly different to one another ($P<0.05$; 2-way repeated measures ANOVA).

In the current study, we investigated the effects of the above mentioned COX inhibitors on the spontaneous contractions occurring in the upper urinary tract of the guinea-pig and rat. In all regions of the guinea-pig upper urinary tract, indomethacin ($\geqslant 10$ μM) and NS-398 ($\geqslant 10$ nM) caused a significant decrease in the frequency of contractions, and hence a reduction in the calculated MIs, whilst VSA had no effect. In the rat renal pelvis, the force of contractions was affected to a greater degree than the frequency of contractions in the presence of the COX inhibitors. VSA increased the force of contractions in both regions of the rat renal pelvis and indomethacin decreased the amplitude of contractions in the proximal ($\geqslant 30$ μM) and distal ($\geqslant 10$ M) renal pelvis. NS-398 had no effect on any of the parameters measured in the two regions of the rat upper urinary tract. Thus the endogenous release of PGs maintains the spontaneous contractions in the upper urinary tract of both the guinea-pig and rat. These results, however, further suggest that COX-2 produces the PGs that regulate contractility in the guinea-pig, while the COX-1 isoform is the primary enzyme involved in sythesizing the PGs in the rat upper urinary tract.

It is well established that PGs can have either an excitatory or inhibitory action on smooth muscle contractility of the upper urinary tract, depending on the type and concentration of the prostanoid, the nature of the tissue and the species involved (Johns & Wooster, 1974; Karmazyn & Dhalla, 1982).

PGE_1 and PGE_2 have both been shown to cause either a decrease or have little effect on the spontaneous activity in human and guinea-pig ureter, whilst $PGF_{2\alpha}$ increases contractility (Johns & Wooster, 1974; Abrams & Feneley, 1975; Forman *et al*., 1978). Vermue & Den Hertog (1987) reported that the smooth muscle of the guinea-pig ureter was insensitive to $PGF_{2\alpha}$. On the other hand, Zhang & Lang (1994) showed that dinoprost (a stable $PGF_{2\alpha}$ analogue) restored contractility in the guinea-pig renal pelvis after indomethacin blockade. The amplitude and frequency of spontaneous contractions in the renal pelvis and ureter of sheep, rabbit and man, are also reduced in a concentration-dependent manner by indomethacin (Angelo-Khattar *et al*., 1985; Thulesius *et al*., 1987; Kimoto & Constantinou, 1991). SC-19220, an EP receptor antagonist (EP_1), blocked contractions of the rabbit renal pelvis, suggesting an excitatory role for PGE_1 and/or PGE_2. In contrast, SC-19220 had no effect on the force of spontaneous contractions in the pelviureteric junction. There was however an increase in the force of spontaneous contractions when non-specific COX inhibitors were used, suggesting that EP_1 receptors don't play a role in the response of this region (Kimoto & Constantinou, 1991). In the present study, we have also demonstrated that indomethacin caused a decrease in the contractility of the upper urinary tract in both the guinea-pig and rat. In the rat, the application of indomethacin ($\geqslant 1$ μM) significantly decreased the amplitude of contractions in both the proximal and distal renal pelvis, which resulted in an overall decrease in the calculated MI. In the guinea-pig upper urinary tract, although indomethacin significantly decreased the frequency of contractions and hence the MIs, varying effects on the amplitude of contractions were observed. The force of contractions were significantly increased, decreased or remained unchanged, in the proximal and distal renal pelvis and the ureter, respectively.

In the guinea-pig upper urinary tract, the application of NS-398 caused a decrease in the MI, arising mainly from a reduction in the frequency of contractions. In contrast, VSA had no effect. It is somewhat surprising that COX-2 appears to be the primary enzyme involved in synthesizing the PGs in the guinea-pig upper urinary tract. Given that COX-1 is thought to be constitutively expressed and involved in maintaining normal cellular processes, one might have expected that COX-1 would be the more likely candidate for maintaining contractility in this whole mount preparation. However, similar findings in isolated preparations of the guinea-pig trachea have been reported previously (Charette *et al*., 1995). In the trachea, both NS-398 and indomethacin were reported to be equipotent in their ability to reduce muscle tone. Western blotting analysis of the tracheal smooth muscle also indicated the presence of the COX-2 enzyme, but not COX-1, suggesting that COX-2 was the primary enzyme involved in producing the PGs which maintain tracheal tone (Charette *et al*., 1995). It may be suggested that COX-2 activity is being induced, due to the trauma of removing and dissecting free the trachea or upper urinary tract. However, this seems unlikely in the present study, as NS-398 did not affect the rat upper urinary tract, despite identical techniques being employed to dissect out the tissues.

In contrast to the guinea-pig, the addition of the COX-1 inhibitor, VSA, to the rat upper urinary tract caused an increase in the force of the spontaneous contractions, while NS-398 was without effect on any of the three parameters measured. These data suggest that in the rat, COX-1 synthesises PGs, which, in turn exert a tonic inhibitory effect. Such interspecies differences in the expression of the two COX isoforms, have previously been reported in the gastrointestinal tract of the rat, dog, human, squirrel monkey and rhesus monkey, whereby the quantity of COX-1 and COX-2 expression differs between species but also between regions within the one species (Kargman *et al*., 1996). Such species-related differences may also be occurring in the present study between the guinea-pig and the rat upper urinary tract.

Another explanation of the varied actions of COX inhibitors on the guinea-pig and rat upper urinary tracts may be the transduction pathway activated by the endogenously-released PGs. To date, the mechanisms by which PGs maintain contractility in the upper urinary tract, have not been fully characterised. However, there is increasing evidence that PGs can have a direct action on capsaicin-sensitive primary afferents, enhancing the release of neuropeptides (Hingtgen & Vasko, 1994; Hingtgen *et al*., 1995). In the bladder of the rat, both PGE_2 and thromboxane B_2 induced rhythmic contractions (micturition reflex) in a manner blocked by hexamethonium or systemic capsaicin desensitisation. Application of indomethacin also significantly increased both the volume and pressure threshold for the reflex micturition, in a manner similar to the effects of systemic capsaicin desensitization. Thus, endogenous PGs released during the distension of the bladder were sensitizing the capsaicin-sensitive nerves, which in turn, facilitated the reflex micturition (Maggi *et al*., 1988; Maggi, 1992). In the rat ureter, distension caused an increase in the afferent nerve activity which, in turn, resulted in a decrease in the efferent renal nerve activity of the contralateral control kidney. This reflex has also been shown to involve capsaicin-sensitive nerves, as it is blocked by capsaicin pretreatment, and mimicked by the application of substance P. Moreover, the synthesis and release of PGs was thought to be involved as perfusion of the animal with indomethacin abolished the increase in afferent nerve activity, while the application of PGE_2 to the renal pelvis perfusate in indomethacin-treated kidneys restored the reflex responses to mechanical stimuli (Kopp & Smith, 1991, 1993; Kopp, 1993).

To date, there have been no investigations to indicate that PGs modulate contractility in the upper urinary tract through the stimulation of capsaicin-sensitive primary afferents. However previous results have indicated species-related differences in the content of neuropeptides stored within the capsaicin-sensitive sensory nerves (Maggi *et al*., 1986, 1987, 1992; Amann *et al*., 1988). If PGs synthesised in the upper urinary tract of the guinea-pig and rat, are indeed sensitising the capsaicin-sensitive nerves, causing a release of neuropeptides and hence modulation of contractility, this may be a factor influencing the different effects of COX inhibitors seen between the two species. Such speculations require further experimentation.

In conclusion, spontaneous myogenic contractile activity in the upper urinary tract of both the guinea-pig and rat is maintained through the endogenous release of PGs. The mechanisms through which this occurs appears to be species related with COX-2 being predominantly involved in synthesising the PGs in the guinea-pig upper urinary tract, whereas, COX-1 is the primary enzyme involved in the rat.

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