Effects of intrinsic prostaglandins on the spontaneous contractile and electrical activity of the proximal renal pelvis of the guinea-pig

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1 The effects of blocking prostaglandin biosynthesis with indomethacin on the spontaneous electrical and contractile activity recorded in smooth muscle strips of the guinea-pig renal pelvis were examined using standard tension and membrane potential recording techniques.

2 Circumferentially cut strips of proximal renal pelvis contracted more frequently ($4.5 \pm 0.2 \text{ min}^{-1}$) than strips cut from the mid region (1.3 ± 0.2 ; $P < 0.05$, $n = 5$) of the renal pelvis.

3 Indomethacin (1 nM – $10 \text{ }\mu\text{M}$) reduced the amplitude and frequency of the contractions of the renal pelvis in a concentration- and time-dependent manner. Contractions were completely abolished in the presence of $30 \text{ }\mu\text{M}$ indomethacin.

4 After indomethacin blockade, activation of prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) receptors with dinoprost (1 – 100 nM) restored the amplitude and frequency of the spontaneous contractions of renal pelvis. Higher concentrations of dinoprost ($> 100 \text{ nM}$ – $3 \text{ }\mu\text{M}$) increased the contraction amplitude of the proximal and mid renal pelvis 1.9 and 1.6 times respectively. The contraction frequency of the mid renal pelvis, but not the proximal pelvis, was also raised above its pre-indomethacin frequency.

5 The spontaneous electrical activity recorded in proximal strips of the renal pelvis was designated to come from three cell types: (i), pacemaker cells (10% of cells recorded), with simple action potentials comprising relatively slow rising and repolarizing phases triggered on top of a slowly-developing pre-potential; (ii), driven cells (75% of cells), with complex action potentials comprising a rapid initial spike, followed by a period of membrane oscillation and a plateau of 0.2–2 s duration; and (iii), intermediate cells (15%) which fired action potentials with an initial rapid and a long plateau phase.

6 Indomethacin (10 – $30 \text{ }\mu\text{M}$) decreased the amplitude and frequency of the action potentials recorded in driven and intermediate cells. The membrane potential of these cells also depolarized 5 mV to $-51.2 \pm 2.6 \text{ mV}$ ($n = 5$).

7 Dinoprost (300 nM – $1.5 \text{ }\mu\text{M}$) increased the rate of action potential discharge, without affecting the membrane potential of driven cells previously exposed to indomethacin ($30 \text{ }\mu\text{M}$).

8 These data suggest that the endogenous release of prostaglandins is necessary for the *in vitro* spontaneous contractile activity recorded in the guinea-pig renal pelvis. Blockade of the synthesis of these prostaglandins appears either to modify the ability of the driven regions of the renal pelvis to fire action potentials or to reduce the coupling of these driven regions to their pacemaker cells.

Keywords: Renal pelvis; action potentials; prostaglandins; indomethacin

Introduction

Since the early 1970's, interest has been focused on the autocrine and paracrine actions of locally-generated prostanoids released from a number of hollow organs following distension, mechanical injury, inflammation, bacterial infection or tissue dissection (Smith *et al.*, 1991). In blood vessels (Parkington *et al.*, 1993) and the gastrointestinal tract (Bauer *et al.*, 1985), the net effect of locally-released prostanoids is generally inhibitory, while in the urinary tract they have an overall excitatory action. For example, the prostanoid modulation of reflex micturition, is thought to arise from an excitation of the bladder smooth muscle by distension-released prostanoids, acting upon capsaicin-sensitive sensory and sympathetic nerves (Maggi, 1992). In the upper urinary tract, application of non-steroidal anti-inflammatory drugs, which block prostaglandin synthesis, have been used in the management of renal colic. The pain of renal colic associated with pelviureteral distension arises from the increased pelviureteral pressure and renal blood flow generated after ureteric obstruction. Indomethacin is thought to induce renal vasoconstriction, which, in turn, reduces glomerular capillary pressure and subsequently pelviureteral pressure.

Recently, indomethacin has been shown to have a more direct action on the contractile activity of the upper urinary tract. The amplitude and frequency of the spontaneous contractions of isolated renal pelvic and ureteral strips from sheep (Thulesius & Angelo-Khattar, 1985; Thulesius *et al.*, 1986) and man (Angelo-Khattar *et al.*, 1985; Zwergel *et al.*, 1990) are reduced by indomethacin in a concentration-dependent manner. In the proximal renal pelvis of the rabbit, only the amplitude of the spontaneous contractions decreased in the presence of indomethacin, acetylsalicylic acid or the prostaglandin antagonist, SC-19220 (Kimoto & Constantinou, 1991); the frequency of their contractions increased. It seems likely that a number of excitatory and inhibitory prostaglandins/cyclo-oxygenase products are being released *in vitro*, the balance of their actions supporting normal pelviureteric peristalsis (Thulesius *et al.*, 1986; Kimoto & Constantinou, 1991). We have investigated the effects of blocking prostanoid synthesis on the spontaneous contractile and electrical activity recorded in the smooth muscle cells of the proximal renal pelvis of the guinea-pig. The amplitude and frequency of these spontaneous contractions were inhibited by indomethacin in a manner correlated with a reduction of the amplitude, duration and frequency of the spontaneous action potentials recorded intracellularly. The decrease in the

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frequency of the action potential discharge arose from the increased number of 'failures' of the 'driven' regions of the renal pelvis to fire action potentials upon being invaded by a 'pacemaker' potential. A preliminary account of these results has been presented previously (Zhang *et al.*, 1994).

Methods

Guinea-pigs (300–600 g) (Monash Animal House strain) were stunned, bled and the kidneys excised. The renal pelvis was exposed by careful dissection of the parenchyma and surrounding adipose/connective tissue, opened up longitudinally and the resultant sheet of tissue pinned in a dissecting dish containing pre-oxygenated physiological saline (see below) at 22°C. Circumferentially-cut strips (2 mm wide; 10–15 mm long) were cut from the proximal and mid portions of the renal pelvis and suspended in a 2 ml organ bath (at 35°C). One end of each strip was fixed at the bottom of the organ bath, the other end was attached to a Grass force displacement transducer (Grass FTO3C) connected to a Grass Polygraph (Model 79D).

Electrophysiological recordings

Intracellular recordings were only made from strips (5 mm wide; 5 mm long) of proximal renal pelvis pinned, serosal side up, in a electrophysiological recording chamber on an inverted microscope. Cells were impaled with fine borosilicate glass microelectrodes which had resistances of 50–150 M Ω when filled with 2 M KCl. Membrane potentials were recorded with a standard unity-gain pre-amplifier, stored on a video cassette recorder using a digital data recorder (List VR-10) and later digitized using a Labmaster analog-to-digital interface, a personal computer (NEC Powermate 286 Plus) and Axotape 1.1 (Axon Instruments) software. Tests for significant changes of contraction and action potential parameters during an experiment were made with Student's paired *t* tests.

Intramural nerves were stimulated with silver-silver chloride wires placed on either side of the muscle strips and connected to a Grass (S88) stimulator. Stimulating parameters (pulses 0.3 ms in duration, 100–150 V at 20–50 Hz for 1–10 s) were chosen to reduce the likelihood of direct muscle stimulation.

Physiological saline

The bicarbonate buffered physiological saline contained (mM): Na⁺ 146, K⁺ 5, Ca²⁺ 2.5, Mg²⁺ 1, Cl⁻ 132, HCO₃⁻ 25, H₂PO₄⁻ 1 and glucose 11. This solution had a pH of 7.3–7.4 after being bubbled with 95% O₂:5% CO₂.

Drugs

Dinoprost trometamol (Upjohn), carbachol (carbamylcholine chloride), atropine sulphate, guanethidine sulphate, histamine dihydrochloride, 4-aminopyridine, cyclopiazonic acid, ouabain, tetrodotoxin (TTX) (all Sigma) and tetraethylammonium (TEA; Eastman Kodak) were freshly prepared in distilled water as stock solutions (0.1–10 mM). Indomethacin and nifedipine were dissolved in ethanol. Drugs were diluted with physiological saline to their final concentrations indicated. Solutions were vigorously bubbled before use to restore any changes in pH.

Results

Tension studies

Proximal and mid portions of the renal pelvis developed regular spontaneous contractile activity after 30–60 min

equilibration in the organ bath and maintained these contractions for 2–4 h. We have confirmed that the frequency of the spontaneous contractions of the proximal strips of the renal pelvis ($4.5 \pm 0.2 \text{ min}^{-1}$) is significantly greater than those recorded in the mid ($1.3 \pm 0.2 \text{ min}^{-1}$; $P < 0.05$, $n = 5$ preparations) (Figure 1) or more distal portions of the pelvis (Constantinou *et al.*, 1977; Kimoto & Constantinou, 1991).

In Figure 1a, it can be seen that indomethacin (300 nM–3 μM) induced both a time- and concentration-dependent reduction in the amplitude and frequency of the spontaneous contractions of the proximal and mid portions of the renal pelvis. To quantify the effects of indomethacin (300 nM–30 μM), the amplitude and frequency of the spontaneous contractions were measured after 20 min exposure to each concentration of indomethacin. The amplitude of the contractions of both the proximal and mid renal pelvis were equally affected by indomethacin (300 nM–3 μM) (Figure 1b). The frequency of these contractions, however was only reduced at indomethacin concentrations $> 1 \mu\text{M}$. When expressed as a fraction of the control, 3 μM indomethacin reduced the frequency of contractions of proximal and mid renal pelvis by 16% and 12% respectively, whereas 10 μM indomethacin induced a 52 and 77% reduction respectively (Figure 1c). Indomethacin, 30 μM , was required to block (within 20 min) the contractions of the renal pelvis completely. Rhythmic contractions, but of reduced amplitude, were restored within 20–30 min following removal of indomethacin from the bathing solution.

Repetitive intrinsic nerve stimulation (50 Hz for 2 s) has a positive inotropic effect on the amplitude and frequency of contraction of both the proximal and mid regions of the renal pelvis (Figure 2a). These positive inotropic effects, which peaked after 30–120 s and slowly declined over 10 min, were still observed after blockade of all spontaneous contractile activity with indomethacin (30 μM) (Figure 2b(i), (ii)). These effects of nerve stimulation were unaffected by the addition of atropine (3 μM) plus guanethidine (3 μM) (Figure 2c) and mostly abolished by the previous application of TTX (3 μM) (Figure 2d) (Maggi *et al.*, 1992; Maggi & Giuliani, 1992).

Figure 1 Effects of indomethacin (300 nM–30 μM ; $\uparrow$) on the amplitude (b) and frequency (c) of the spontaneous contractions generated by circumferentially-cut strips ($n = 5$) of the proximal (a(i), b,c, $\circ$) and mid (a(ii), b,c, $\bullet$) renal pelvis of the guinea-pig.

Effects of dinoprost

Addition of dinoprost (1 nM–3 μ M), a stable analogue of PGF_{2 α} restored the spontaneous contractile activity of the proximal and mid portions of the renal pelvis abolished by indomethacin (30 μ M) (Figure 3a). The proximal strips of renal pelvis appeared more sensitive to the action of dinoprost, contractions being activated with dinoprost concentrations >1 nM. In contrast, the mid renal pelvis contracted at dinoprost concentrations >10 nM (Figure 3). Dinoprost (1 nM–3 μ M) was equally effective at increasing the contrac-

tion amplitude (expressed as % of the maximum induced by dinoprost) of the proximal and mid renal pelvis (Figure 3b). Their averaged maximal contractions to dinoprost (3 μ M), however, were significantly larger (425 $\pm$ 23 and 327 $\pm$ 33 mg respectively) than their control contractions (230 $\pm$ 59 and 200 $\pm$ 36 mg; P < 0.05, n = 4) in the absence of indomethacin. Interpolation of the four individual concentration-response curves showed that the average concentrations of dinoprost to restore the spontaneous contractions of the proximal and mid portions of the renal pelvis to their pre-indomethacin amplitudes were 148 $\pm$ 47 and 425 $\pm$ 56 nM

Figure 2 Effects of intramural sensory nerve stimulation (50 Hz for 2 s; ●) on the spontaneous contractile activity of the proximal (i) and mid (ii) renal pelvis of the guinea-pig in the absence (a) and after 20 min exposure (b) to 30 μ M indomethacin. These positive inotropic effects of nerve stimulation in the presence of indomethacin were not prevented by atropine (3 μ M) + guanethidine (3 μ M) (c) but were blocked by tetrodotoxin (TTX 3 μ M, d).

Figure 3 Stimulation of prostaglandin F_{2 α} receptors with dinoprost (1 nM–3 μ M) induced spontaneous contractions in both proximal (a(i),b,c, ○) and mid (a(ii),b,c, ●) portions of the renal pelvis (n = 4) previously blocked with indomethacin (30 μ M); increasing both their strength (b) and frequency (c) of contraction in a concentration-dependent manner.

respectively ($P < 0.05$, $n = 4$). The concentrations of dinoprost required to restore the frequency of contraction of the proximal and mid renal pelvis to their pre-indomethacin frequencies were 102 ± 56 and 63 ± 24 nM ($P > 0.05$). Higher concentrations (> 100 nM) of dinoprost could not, however, increase the contraction frequency of the proximal strips to a significantly greater extent than the control frequency before the addition of indomethacin (4.5 ± 0.2 min $^{-1}$; $P > 0.05$, $n = 4$) (Figure 3c). In contrast, these concentrations of dinoprost (> 100 nM) significantly increased the contraction frequency of the mid renal pelvis to approximately two fold greater than its pre-indomethacin frequency (1.5 ± 0.1 min $^{-1}$; $P < 0.05$, $n = 4$) (Figure 3c).

Dinoprost (> 100 nM) also induced a small tonic contraction in the proximal, but not the mid renal pelvis in both the absence and presence of indomethacin ($n = 11$). These tonic contractions were 5–40% of the amplitude of the control spontaneous contractions in the absence of indomethacin. Tonic contractions in the presence of indomethacin were also not affected by nifedipine (1 μ M) ($n = 10$), nicardipine (1 μ M) ($n = 2$), ouabain (10 μ M) ($n = 3$) or cyclopiazonic acid (10 μ M) ($n = 4$), but were blocked by Cd $^{2+}$ (0.5 mM) ($n = 5$) or upon removal of Ca $^{2+}$ from the bathing solution ($n = 7$) (data not shown). These data suggest that dinoprost may also be triggering an influx of Ca $^{2+}$ through receptor-operated pathways that do not involve dihydropyridine-sensitive Ca $^{2+}$ channels, the Na-K pump or the release of Ca $^{2+}$ from internal stores. Spontaneous contractions of both proximal and mid renal pelvis after indomethacin-induced blockade could also be triggered with the stable PGI analogue, iloprost (100 nM) ($n = 3$) and with histamine (10 μ M) ($n = 1$). Exposure to 20 mM K $^{+}$ saline has both positive inotropic and chronotropic effects on the spontaneous contractions of the proximal and mid renal pelvis (Cole *et al.*, 1988); exposure to 100 mM K $^{+}$ induced a tonic contraction of 100–130% the amplitude of the spontaneous contractions in control saline. These contractions to high K $^{+}$ saline were not affected by 30 μ M indomethacin (applied for > 20 min), but were abolished by nifedipine (1 μ M) ($n = 4$).

Microelectrode studies

To date, only a few preliminary electrophysiological recordings have been obtained from the renal pelvis, using the sucrose gap (Zawalinski *et al.*, 1975) or intracellular microelectrode (Golenhofen & Hannappel, 1974; Seki & Suzuki, 1990; Tsuchida & Suzuki, 1992) techniques. We have recently presented an extensive study of the properties of the action potentials recorded in the guinea-pig proximal renal pelvis recorded with intracellular microelectrodes (Zhang & Lang, 1993). Briefly, three types of spontaneous action potential discharge were recorded: (i), 10% of cells ($n > 100$) ('pacemaker' cells) had an action potential with a simple 'pacemaker-like' waveform which was triggered on top of a slowly-developing prepotential. These cells had membrane potentials, measured 600 ms preceding a spontaneous event, of -40 to -50 mV (-46 ± 2 mV, $n = 7$) (Figure 4a); (ii) 75% of cells ('driven' cells) had more complex action potentials consisting of an initial spike, followed by a period of membrane oscillation and a plateau (0.2–2 s in duration), and membrane potentials of -55 to -60 mV (-58 ± 2 mV, $n = 14$) (Figure 4c,d); (iii) the remaining 15% of cells fired action potentials with a single initial spike and plateau and had membrane potentials of about -45 mV ('intermediate' cells) (Figure 4b) (Zhang & Lang, 1993). Cells from groups (ii) and (iii) had distinct refractory periods, often failing to trigger an action potential 5–20 s after a preceding one. During such failures, the underlying triggering pacemaker potential was revealed (Figure 4b,c,d). Action potentials in all three cell types were blocked upon addition of nifedipine (1–10 μ M) (Figure 6a(iv), b(iv)) ($n = 6$) or Cd $^{2+}$ (100 nM) ($n = 3$), blockers of voltage-operated Ca $^{2+}$ channels.

Figure 4 Spontaneous action potentials recorded in strips of proximal renal pelvis were divided into three general types: (a), 10% of cells ($n > 100$ cells) had membrane potentials of -40 to -45 mV (measured 600 ms before each action potential), fired simple action potentials and were thought as being recorded from 'pacemaker' cells; (c,d), 75% of cells had membrane potentials of -55 to -60 mV, fired complex action potentials with a rapidly-rising initial spike which was followed by a period of membrane oscillation and a plateau phase were designated as 'driven' cells; (b), the remaining cells (15%) had action potentials with an initial spike and plateau were designated as 'intermediate' cells.

Effects of indomethacin

Figure 5 illustrates both the time- and concentration-dependent effects of indomethacin (15–30 μ M) on the membrane potential and action potentials recorded in a driven cell of the proximal renal pelvis. Both the amplitude and frequency of these action potentials decreased with indomethacin (15–30 μ M) (Figure 5a) and this decrease was associated with a time-dependent membrane depolarization of 5 mV to -51.2 ± 2.6 mV (control membrane potential -56.8 ± 3.3 mV; $P < 0.05$, $n = 5$). In five cells, the averaged frequency, initial spike amplitude and half-amplitude duration were respectively 1.6 ± 0.9 min $^{-1}$, 17.0 ± 7.3 mV and 307 ± 179 ms after a 20 min exposure to indomethacin (30 μ M); significantly ($P < 0.05$) smaller than their control values of 6.7 ± 1.6 min $^{-1}$, 34.3 ± 1.2 mV and 1266 ± 307 ms respectively (Figure 5b). The decrease in action potential frequency arose from the increase in 'failures' to fire an action potential, as the frequency of the underlying pacemaker potentials was little affected (Figure 5a(i), (iv) (v)). These effects of indomethacin were reversible after 30 min washout, although the duration and amplitude of the action potentials never fully recovered (data not shown).

Dinoprost, 300 nM and 1.5 μ M, significantly increased the rate of action potential discharge to 7.8 ± 2.9 and 14 ± 5.1 min $^{-1}$ respectively in non-pacemaker cells previously slowed with 30 μ M indomethacin (1.6 ± 0.9 min $^{-1}$; $P < 0.05$, $n = 5$). In the intermediate cell illustrated in Figure 6a,b, this acceleration of action potential discharge with dinoprost (1.5 μ M) was associated with a membrane depolarization of some 10 mV and possibly the recruitment of more than one

Figure 5 Effects of indomethacin (Indo; 15–30 μM) on the spontaneous action potential recorded in a driven cell of the proximal renal pelvis. Spontaneous action potential discharge became irregular (a(ii),(iii)) with time in indomethacin (15 μM); their frequency of discharge decreasing from 6.7 ± 1.6 to $1.6 \pm 0.9 \text{ min}^{-1}$ ($P < 0.05$, $n = 5$) in 30 μM indomethacin (a(iv),(v)). The pacemaker potentials underlying these action potentials could be clearly recorded in the presence of indomethacin, their frequency appeared little affected (a(i),(v)). The change in time course of the action potentials in indomethacin (15–30 μM) has been illustrated by superimposing a control action potential on action potentials recorded after 5 (b(i)) and 10 (b(ii)) min exposure to 15 μM indomethacin and after 5 (b(iii)) and 10 (b(iv)) min exposure to 30 μM indomethacin. The averaged initial spike amplitude and half-amplitude duration was respectively $17.0 \pm 7.3 \text{ mV}$ and $307 \pm 179 \text{ ms}$ after 30 min exposure to indomethacin (30 μM), compared with their control values of $34.3 \pm 1.2 \text{ mV}$ and $1266 \pm 307 \text{ ms}$ ($P < 0.05$; $n = 5$).

pacemaker potential. In contrast, the membrane potential of driven cells (Figure 6c) was -51.5 ± 6.7 ($n = 3$) and -49.2 ± 4.2 ($n = 5$) respectively after 5 min exposure to 300 nM and 1.5 μM dinoprost (control membrane potential in 30 μM indomethacin of $-51.2 \pm 2.6 \text{ mV}$) (Cole *et al.*, 1988).

Nerve stimulation (50 Hz for 2–10 s) also induced action potential discharge in non-pacemaker cells exposed to indomethacin (30 μM) (Figure 7a,b). In the driven cell illustrated in Figure 7, the action potentials initiated upon nerve stimulation (Figure 7b) have been compared with those initiated by dinoprost (1.5 μM ; Figure 7c). The action potentials initiated by nerve stimulation were slightly briefer and associated with a membrane after-hyperpolarization (Figure 7b), which was not evident in dinoprost. The increase in the frequency of action potential discharge in the presence or absence of indomethacin were abolished by the previous application of TTX (1.6 μM ; $n = 6$). Together with the contraction data, it is clear that the mechanisms which control transmitter release are not affected by indomethacin. Also, the mechanism/conductance changes which prevent action potential generation in the presence of indomethacin can be reversed rapidly (within 30 s) upon nerve stimulation or $\text{PGF}_{2\alpha}$ receptor activation.

Discussion

The renal pelvis in man and many mammals consists of two morphologically and histochemically distinct types of smooth

muscle cells. Typical smooth muscle cells are spindle shaped and grouped into bundles, which branch and interconnect with adjacent bundles, to form a continuous muscle layer from the major and minor calyces to the renal pelvis, the pelviureteric junction and into the ureter. 'Atypical' smooth muscle cells form a thin layer of muscle cells, internal to the typical cells, which extends from the calyceal-renal parenchyma attachment to the pelviureteric junction. Few of these cells appear in the pelviureteric junction and they are generally absent in the proximal ureter. These atypical smooth muscle cells are characterized by their irregular shapes and cell contents, as observed under the electron microscope, and by their lack of non-specific cholinesterase (Gosling & Dixon, 1972; 1974). They have areas of close apposition with other atypical cells and with bundles of typical smooth muscles so that it is likely that they, like the interstitial cells of the gastrointestinal tract, form specialized preferential pathways of electrical conduction. The intrinsic electrical activity of the most proximal atypical cells has been thought to drive the electrical and contractile activity throughout the renal pelvis and therefore ureteric peristalsis (Gosling & Dixon, 1972; 1974; Hannappel & Lutzeyer, 1978).

The nature of the electrical activity in the ureter has been extensively studied using extracellular and intracellular recording techniques (Kuriyama *et al.*, 1967; Kuriyama & Tomita, 1970; Shuba, 1981) and more recently at the whole-cell and single channel current level (Imaizumi *et al.*, 1989; 1990; Lang, 1989; 1990). Recently, we have presented evidence detailing the nature of the ionic conductances underlying the

Figure 6 Stimulation of prostaglandin $F_{2\alpha}$ receptors with dinoprost (300 nM–1.5 μ M) restores the frequency of action potential discharge in intermediate (a(iii), b(iii)) and driven cells (c(iii)) cells previously reduced by indomethacin (Indo; 30 μ M (a(ii), b(ii), c(ii))); the amplitude and half-amplitude duration of the action potentials in dinoprost never fully recovered (b(iii), c(iii)). The intermediate cell illustrated in (a) has been exposed to indomethacin (30 μ M) for 30 min. In b(i)–(iv) the action potentials highlighted in (a) have been replotted on an expanded time scale. Note the membrane depolarization in the presence of nifedipine (1 μ M) (a(iv), b(iv)).

electrical activity in the renal proximal pelvis (Zhang & Lang, 1993). We have divided the recorded electrical activity into three distinct groupings and have speculated on their physiological roles. The cells with the simple action potentials have been termed 'pacemaker cells', analogous with the heart. These pacemaker action potentials were generally recorded in cells near the urothelial border, suggesting that they were made in atypical cells as has often been proposed (Gosling & Dixon, 1972; 1974; Hannappel & Lutzeyer, 1978). The cells with the longer complex action potentials, similar to those recorded in the ureter (Kuriyama *et al.*, 1967; Kuriyama & Tomita, 1970; Shuba, 1981), have been termed 'driven cells', and are distinguished by their more negative membrane potentials (–55 to –60 mV cf. –45 mV in pacemaker cells). The remaining were designated 'intermediate cells'. We would like to suggest that the pacemaker action potentials were recorded from atypical smooth muscle cells, while the action potentials recorded from intermediate and driven cells were recorded from typical smooth muscle cells which were respectively in close and far electrical contact with a pacemaker region.

In the present experiments, we have demonstrated that the blockade of endogenous prostanoid synthesis leads to an inhibition of both the frequency and amplitude of the spontaneous contractions of the proximal and mid renal pelvis of the guinea-pig. The concentrations of indomethacin (5–30 μ M) used in the electrophysiological experiments, while relatively higher than the 50% inhibitory concentrations (IC_{50}) required to block prostaglandin synthesis in homogenates or subcellular fractions of brain, lung, spleen or kidney (0.2–3.7 μ M) (Flower, 1974), are comparable to the indomethacin concentrations used to block prostaglandin synthesis in the urinary (Kimoto & Constantinou, 1991; Thulesius &

Angelo-Khattar, 1985; Thulesius *et al.*, 1987; Cole *et al.*, 1988) and gastrointestinal tracts (Bauer *et al.*, 1985), perfused lungs and hearts and in rat isolated uteri (Flower, 1974). We felt it important that we maintained our electrode impalements throughout the onset of indomethacin action so that the action potentials recorded in control saline could be directly compared with those in indomethacin. Given the difficulty of maintaining these impalements in spontaneously contracting preparations and the slower onset of action of lower concentrations of indomethacin (>150 min at 1 μ M indomethacin) (Angelo-Khattar *et al.*, 1985; Thulesius & Angelo-Khatta, 1985), we chose concentrations of indomethacin (5–30 μ M) which produced steady-state blockade within 20 min.

During the onset of indomethacin (10–30 μ M) action (Figure 5), the action potentials recorded in driven and intermediate cells reduced in amplitude and duration, consistent with the reduced contraction amplitudes observed with lower concentrations (<1 μ M) of indomethacin and with the time-dependent decrease observed with higher concentrations of indomethacin (Figure 1). Final blockade of muscle contraction by indomethacin was associated with a loss of action potential discharge. The membrane depolarization in the presence of indomethacin, we believe, arises from the reduction of the time-dependent K^+ conductances associated with the downstroke and refractory periods of each action potentials. With fewer action potentials of shorter amplitude and duration discharging, these K^+ conductances would have less influence and the membrane potential would therefore be expected to settle closer to its 'true' membrane potential. A similar membrane depolarization to near –40 mV is recorded in driven, intermediate and pacemaker cells when action potential discharge is blocked with nifedipine (1 μ M) (Figure

Figure 7 Nerve stimulation (NS; 50 Hz for 10 s) in the presence of indomethacin (30 μ M) (a,b) induced action potential discharges of a similar time course to those induced by dinoprost (1.5 μ M), except for a larger after-hyperpolarization (a,c). (b,c) are the action potentials highlighted by the brackets in (a) illustrated on an expanded time scale and superimposed on the membrane potential in indomethacin.

6a iv) (Zhang & Lang, 1993). Alternatively, this membrane depolarization in the presence of indomethacin could perhaps suggest that endogenous prostanoids are constantly activating a K^+ conductance, or turning off a Na^+/Cl^- conductance, which helps to maintain the more negative ('diastolic') membrane potential in the driven and intermediate cells. Recently, however, we have demonstrated that an increase in action potentials discharge is associated with the membrane depolarization induced when membrane K^+ conductances are blocked with tetraethylammonium or 4-aminopyridine (2 mM), or when cationic conductances are activated with carbachol (10 μ M) (Zhang & Lang, 1993).

What causes failure of action potential discharge in driven cells remains uncertain. The prostanoids may well be sustaining the smooth muscle Ca^{2+} channels in an activatable state,

perhaps by shifting the voltage range over which they will open. The loss of this support would reduce the amplitude and duration of the driven action potential which would reduce their associated K^+ conductances leading to membrane depolarization (as discussed above) and further voltage-dependent inactivation of the remaining activatable Ca^{2+} channels. Although, such a decrease in the Ca^{2+} conductance in the presence of indomethacin can be correlated with the time-dependent decrease in the amplitude of the spontaneous contractions, it cannot explain the contractions to raised K^+ concentrations (20–100 mM) in indomethacin. We therefore believe that it is more likely that the prostanoids help to maintain the electrical coupling between pacemaker and driven regions of the renal pelvis. A reduction of this coupling would reduce the likelihood of action potential discharge in a driven cell upon stimulation by a pacemaker potential. The resulting smaller or failed action potentials would, in turn, lead to a more depolarized diastolic membrane potential.

When driven cells failed to discharge an action potential in the presence of indomethacin, the underlying pacemaker trigger was still present at the same frequency as its pre-indomethacin control frequency, suggesting that these pacemaker cells were little affected. As recordings were infrequently made in pacemaker cells (in only nine cells from >100 recorded cells), we have not yet been able to confirm this suggestion directly. Support of this notion, however, comes from the fact that we could not increase the frequency of the spontaneous contractions of the proximal renal pelvis above its intrinsic frequency before indomethacin inhibition. Also, it has been previously demonstrated in the sheep ureter that the frequency of its spontaneous contractions was not quantitatively related to the measured concentrations of the spontaneously-released prostanoids ($PGF_{2\alpha}$, 6-keto- PGF_1 and thromboxane B_2) (Thulesius *et al.*, 1987).

Direct activation of driven cells with applied dinoprost (300 nM–1.5 μ M) resulted in a rapid restoration of action potential discharge in a concentration-dependent manner. In only one cell, was this restoration of action potential discharge accompanied with membrane hyperpolarization, as might be expected from the effects of indomethacin. In fact, the membrane potential was unchanged after 5 min exposure to 300 nM dinoprost and depolarized 2 mV in 1.5 μ M dinoprost. These small changes in membrane potential are presumably associated with the Ca^{2+} -dependent tonic contractions of the proximal renal pelvis induced by dinoprost (>100 nM) which were insensitive to dihydropyridine Ca^{2+} channel blockers or depletors of internal Ca^{2+} stores. They must also represent a sizable increase in membrane conductance as they could prevent the after-hyperpolarizations usually associated with each action potential (Figure 7).

In conclusion, we have demonstrated that endogenous prostanoids maintain the smooth muscle contractility of the renal pelvis by maintaining the ability of 'driven' areas to generate action potentials. This may be as a consequence of changes in the membrane conductances in the driven cells, or as a reduction of the coupling between these cells and their pacemaker cells.

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