Prostaglandin E_2 mediates spontaneous rhythmic contraction in rabbit detrusor muscle

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Introduction: *The purpose of this investigation was to determine if prostaglandin E_2 (PGE_2) is produced by rabbit detrusor free of urothelium and demonstrate that PGE_2 is responsible for the generation of spontaneous rhythmic contraction (SRC).*

Methods: *A bioassay was performed in which contraction frequency in strips of rabbit detrusor was compared before and after addition of superfusate from incubating sections of rabbit detrusor. Specificity was determined by testing the effects of SC-51089, a PGE_2 (EP1) antagonist. Effects on development of tension were determined in artery segments after treatment with increasing doses of PGE_2, $PGF_{2\alpha}$, and TXA_2, and a section of femoral artery was used as a negative control. Confirmation of PGE_2 production was then determined using EIA kits.*

Results: *Increased rhythmic frequency was identified after superfusate from a section of rabbit detrusor free of urothelium was added to strips of detrusor from the same animal. Additional experiments demonstrated that rhythmic frequency generated after treatment with PGE_2 was significantly reduced after treatment with SC-51089. In artery smooth muscle, prostaglandin dose response experiments demonstrated that only TXA_2 induced contraction at physiologic doses ($< 10^{-7}M$). As a negative control, subsequent treatment of a section of femoral artery with detrusor superfusate failed to increase tension, confirming a lack of TXA_2 production. EIA confirmed that PGE_2 production increased by 4.8-fold in strips of detrusor free of urothelium after 15 minutes of incubation and that this production was blocked by ibuprofen and a COX-1 inhibitor.*

Conclusions: *Rabbit detrusor produces PGE_2 which is the most likely mediator of SRC.*

Key Words: detrusor, overactive bladder, smooth muscle, prostaglandins

Introduction

In the classic model of the "micturition cycle," there is a completely quiescent filling phase and a dynamic voiding phase. However, this model fails to account for observations of spontaneous activity in the bladder that were originally described in the works of Mosso[1] and Sherrington[2] in the late 19th century. Spontaneous rhythmic contraction (SRC) is a phenomenon common to mammalian bladders that occurs during bladder filling. Although the precise function of SRC is unknown, recent studies suggest it may be important in overactive bladder.[3] Studies demonstrate that bladder specimens from patients with overactive bladder and animals with detrusor overactivity display enhanced SRC.[4] Importantly, SRC cannot be abolished by muscarinic antagonists, does not require an intact urothelium, and does not require intact connections to the central nervous system. Therefore, the phenomenon of SRC is likely an intrinsic property of the detrusor itself.[5,6] Our recent published work demonstrates that specific inhibition of both cyclooxygenase (COX) isoenzymes and certain prostaglandin receptors can abolish SRC in strips of rabbit detrusor free of urothelium, and that PGE_2, PGF_{2a} and TXA_2, can each reestablish SRC.[7]

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It is now evident that the urothelium and suburothelial space play a prominent role in prostaglandin production.[8,9] However, it is not known whether the muscular wall (detrusor) is sufficient for both the production of prostaglandins and the maintenance of SRC. Therefore, the purpose of the current investigation was to determine if prostaglandins are produced by rabbit detrusor free of urothelium and to demonstrate through a bioassay that these detrusor-specific prostaglandins are responsible for the generation of SRC.

Materials and methods

Tissue preparation

All experiments involving animals were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Whole bladders and carotid, femoral and renal arteries from New Zealand White rabbits were removed immediately after euthanization with pentobarbital. Organs were washed, cleaned of adhering tissues, and stored in ice-cold physiological salt solution (PSS). For force and contraction experiments, thin strips (approximately 1 mm x 4 mm) of detrusor were cut from the supratrigonal aspect of the posterior bladder wall following the natural bundling and dissected free of underlying urothelium. In bioassay experiments, the entire anterior bladder (with urothelium, without urothelium, or urothelium only) was used to generate a superfusate (SF) to determine the potential source of prostaglandins. Artery rings were prepared for prostaglandin dose-response curves and the femoral artery was chosen to serve as the bioassay recipient of donor bladder tissue SF.

Solutions and drugs

Tissues were stored in cold (0-4°C) physiologic salt solution (PSS), composed of NaCl, 140 mM; KCl, 4.7 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.6 mM; Na_2HPO_4, 1.2 mM, morpholinopropanesulfonic acid, 2.0 mM (adjusted to pH 7.4 at either 0 or 37°C, as appropriate), Na^2 ethylenediamine tetraacetic acid (EDTA; to chelate trace heavy metals), 0.02 mM; and dextrose, 5.6 mM. High purity (17MΩ) water was used throughout. At the beginning or end of each experiment, each tissue was contracted with KCl (110 mM, substituted isosmotically for NaCl) to measure maximum contraction, and relaxed by incubation in a Ca^{2+}-free solution plus 10 M papaverine to measure minimum tension. Tension responses produced by addition of prostaglandins and donor bladder SF to isolated detrusor strips and artery rings were normalized to these maximum and minimum values and expressed as T/T_o. Ibuprofen, indomethacin, NS-398 (COX-2 inhibitor), SC-560 (COX-1 inhibitor), U-44619 (TXA_2 mimetic), and PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI, USA). $PGF_{2\alpha}$ was purchased from Sigma Aldrich (St. Louis, MO, USA), and SC-51089, a PGE_2 (EP_1) antagonist, was purchased from Biomol International (Plymouth Meeting, PA, USA).

Apparatus

Each tissue was secured by two small clips (detrusor) or pins (artery rings), one of which was attached to a micrometer for manual length adjustments and the other to an isometric force transducer for measurement of tension. Active tension was calculated as the total tension minus the passive tension. Each strip of muscle was incubated in aerated PSS at 37°C in a water-jacketed tissue bath (Radnotti Glass Technology, Monrovia, CA, USA). Voltage signals were digitized (model SCB-68, National Instruments; http://www.ni.com), visualized on a computer screen as force (gm), and stored for later analyses. Initial data analysis was performed through DASYLab (National Instruments; http://www.dasylab.net/dasylab_english) and further processed using commercially available spreadsheets.

Bioassay

An apparatus consisting of four aerated water-jacketed tissue baths containing 37°C PSS was utilized. For each experiment, strips of bladder free of urothelium were placed in baths #1 and #2. Urothelium only or a 2 mm-3 mm section of femoral artery (which ultimately served as a negative control for production of PGE_2, see "Results") was placed in bath #3. The entire anterior side of the bladder either without or with urothelium was hung without tension from a clip in bath #4. For the bioassay acceptor tissues, length at which optimum tension is produced (L_o) was determined following methodology from Speich.[10] Fresh aliquots of 15 ml PSS were then added to baths #1 - #3 and volumes of 20 ml or 30 ml were added to bath #4. Strips in baths #1 and #2 were monitored for return of consistent SRC (usually approximately 60-90 minutes). Strips in baths #1 and #2 were arbitrarily assigned as "control" or "test" strips. Specific COX inhibitors were added to the "test" strip and equal volumes of drug vehicle were added to the "control" strip. Following COX administration, SRC was recorded for 10 minutes. The medium containing the "test" strip was removed and replaced with 15 ml of SF from the donor bladder tissue (bath #4) or urothelium (bath #3) plus COX inhibitor. To determine whether the rhythmic contraction

induced in the acceptor tissue was caused by PGE_2 contained within the donor tissue SF, the ability of the PGE_2 receptor antagonist, SC-51089 (3 µM) to relax the induced rhythm was determined by adding this drug to the "test" tissue bath. A similar protocol was used to test whether femoral artery rings would respond to bladder tissue SF.

Effect of prostaglandins on development of tension in arterial smooth muscle.

To further determine which PG was responsible for generation of SRC in rabbit detrusor, dose-response curves were generated for femoral, carotid, and renal artery segments harvested from the same animals. In these experiments, the degree of tension (T) developed after cumulative addition of increasing doses (10^{-10}M – 10^{-5}M) of PGE_2, $PGF_{2\alpha}$, or TXA_2 were normalized to the maximum tension produced for each tissue by KCl (T_o), and were reported as T/T_o.

EIA assays for prostaglandins (PG) and PGE_2.

To determine the concentration of PG and PGE_2 produced by strips of bladder free of urothelium, the Cayman Prostaglandin Screening EIA Kits 514012 (100% sensitive for PGE_2, $PGF_{2\alpha}$, or TXA_2) and 514010 (specific for PGE_2) were utilized following the manufacturers recommendations. Strips were incubated in aerated 1.0 ml PSS or drug solutions including NS-398 (10^{-7}M), SC-560 (10^{-7}M) and ibuprofen (30 µM) at 37C for 1, 5, and 15 minutes. Drug concentrations were selected based on previous work demonstrating effective suppression of SRC.[7] Relatively low concentrations of the COX-2 and COX-1 inhibitors were used to better demonstrate selectivity. After each time interval, an individual strip was removed and washed in 37°C PSS, the incubation medium was aspirated and stored at -20°C for subsequent EIA analysis, and the strip was placed into a new tissue bath containing fresh PSS for additional time periods. Solutions were run in duplicate on a plate spectrophotometer to obtain average absorbance values corrected for Ellman's reagent (blank). By utilizing a standard curve for each experiment, the concentration of unknown samples was calculated mathematically.

Bradford assays

To account for differences in protein content for individual strips, EIA determined PG or PGE_2 concentrations were normalized to protein content using a Bradford Assay Kit (Thermo Scientific, Rockford, Il, USA) following the manufacturer's instructions. Briefly, after completion of EIA time course experiments, tissues were dried in -80ºC acetone for 1 hour and dry weights were determined. Tissues were homogenized in 1.5 mL lysis buffer plus DTT (75 mg) for 20 minutes and centrifuged for 10 minutes at 14500 rpm. Supernatant was removed, stored at -20°C, and run on a Biomate Spectrophotometer to obtain absorbance values. The protein content of each unknown sample was then determined by comparison to a standard curve.

Statistics

The null hypothesis was examined using Students' t-test (when two groups were compared) or one-way analysis of variance (ANOVA). To determine differences between groups following ANOVA, the Student-Neuman-Keuls post-hoc test was used. In all cases, the null hypothesis was rejected at $p < 0.05$. For each study described, the n value equals the number of bladders.

Results

PG production using a detrusor bioassay.

To determine whether PGs that are produced by rabbit detrusor free of urothelium mediate SRC, a four-step bioassay was performed. In strips of rabbit detrusor without urothelium, SRC developed within approximately 1 hour (step 1: development of SRC; Figure 1a, left side). This SRC was abolished after treatment with 10^{-6} M of the non-selective COX inhibitor indomethacin (step 2: abolishment of SRC; Figure 1a, INDO). Replacement of the medium with SF obtained from donor bladder tissue resulted in restoration of SRC (step 3: restoration of SRC; Figure 1a, right side). Application of 3µM SC-51089, a PGE_2 antagonist, abolished the restored rhythmic contraction, demonstrating that the active substance in the SF is likely PGE_2 (step 4: abolishment of restored SRC; Figure 1a far right side).

The degree of SRC was assessed by counting the number of contractile spikes during the final 2 minutes of each of the four steps. In strips of detrusor free of urothelium (n = 5), SRC frequency averaged ~37 spikes/2 min, and this was reduced to ~1.0 spike/2 min after treatment with indomethacin (Figure 1a, example trace, and Figure 1b, "1" versus "2"). Treatment with SF from detrusor muscle free of urothelium restored average SRC frequency to ~27 spikes/2 min, and this was abolished with SC-51089 (Figure 1a, example trace, and Figure 1b, "3" versus "4"). In another set of detrusor strips free of urothelium (n = 3), SRC frequency averaged ~20 spikes/2 min, and this was abolished by indomethacin (Figure 1c, "1" versus "2"). Addition of SF from

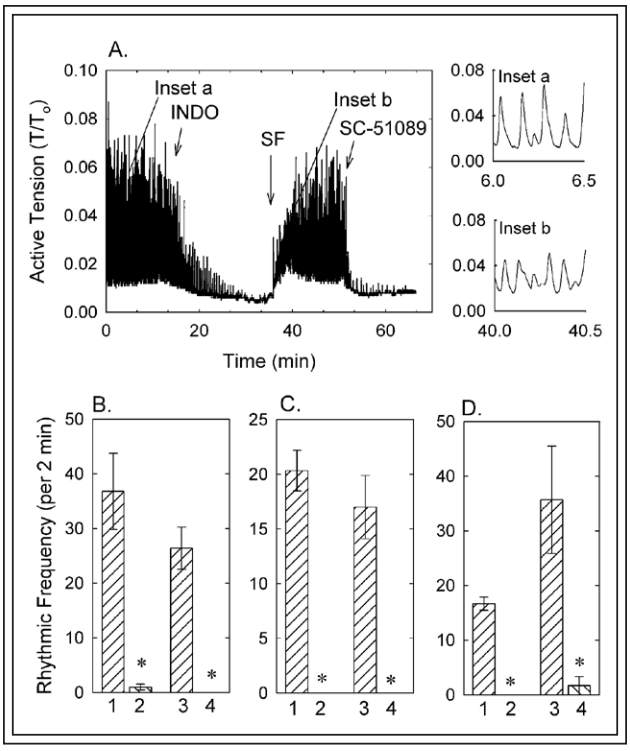

Figure 1. A. Representative 4-step bioassay tracing with superfusate (SF) obtained from rabbit DSM with intact urothelium. Strong spontaneous rhythmic contraction (SRC) is noted after incubation in NPSS (step 1) prior to introduction of $1x10^{-6}$M indomethacin (INDO, step 2) which nearly abolishes rhythm. Addition of superfusate (SF, step 3) from a large section of bladder free of urothelium was introduced and redevelopment of SRC was noted. Subsequent treatment with a PGE_2 antagonist (SC-51089, step 4) at a final concentration of 3μM abolished this redeveloped rhythm. Inset "a" and "b" are utilized to show relative amplitude and frequency of redeveloped rhythm after addition of SF. **B** (n = 5, DSM), **C** (n = 3, DSM + urothelium), and **D** (n = 3, urothelium only) demonstrate the contractile frequency (spikes/2 min) at the end of each bioassay step (1-4). *$p < 0.05$ (step 2 versus 1 and step 4 versus 3).

detrusor muscle with intact urothelium resulted in restored SRC averaged frequency of ~17 spikes/2 min, and this was abolished with SC-51089 (Figure 1c, "3" versus "4"). In a third set of detrusor strips free of urothelium, (n = 3) average SRC frequency was ~17 spikes/2 min, and this was abolished by indomethacin (Figure 1d, "1" versus "2"). Addition of SF from urothelium only (free of detrusor muscle) resulted in a restored SRC average frequency of ~36 spikes/2 min, and this was reduced to ~2 spikes/2 min by SC-51089 (Figure 1d, "3" versus "4").

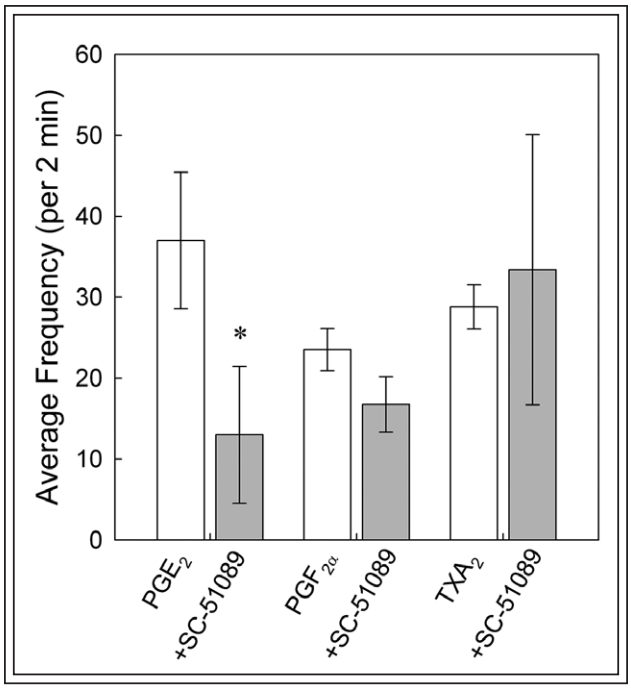

Figure 2. Graphical representation of the frequency of rhythmic contraction (contractions/2 min) of rabbit DSM free of urothelium after the addition of prostaglandin agonists PGE_2, $PGF_{2\alpha}$, and TXA_2 (U-46619) (doses ranging from $3x10^{-9}$M to $3x10^{-8}$M). Agonists were added after SRC was abolished with indomethacin (10^{-6}M) and the contraction frequency was measured (white bars). A PGE_2 (EP1 receptor) antagonist (SC-51089, 3μM) was then added and the contraction frequency was again recorded (gray bars). *$p < 0.05$.

Determination of selectivity of SC-51089 in strips of DSM.

In a similar set of experiments, strips of rabbit detrusor free of urothelium were treated with indomethacin after development of SRC. Strips were then treated with PGE_2, $PGF_{2\alpha}$, or TXA_2 to cause redevelopment of SRC as previously determined.[7] PGE_2, $PGF_{2\alpha}$ and TXA_2 all caused redevelopment of SRC with frequency of ~25 to ~35 spikes/2 min (Figure 2, open bars). The PGE_2 antagonist SC-51089 (3 μM) significantly reduced SRC frequency induced only by PGE_2 (n = 3). That is, although treatment with $PGF_{2\alpha}$ and TXA_2 restored SRC frequency, subsequent treatment with SC-51089 did not reduce the frequency. These results support the conclusion that PGE_2 produced by both detrusor muscle alone and urothelium alone mediates SRC.

Effects of prostaglandins using an artery bioassay

An arterial bioassay was performed to help confirm that PGE_2 was the mediator most responsible for SRC.

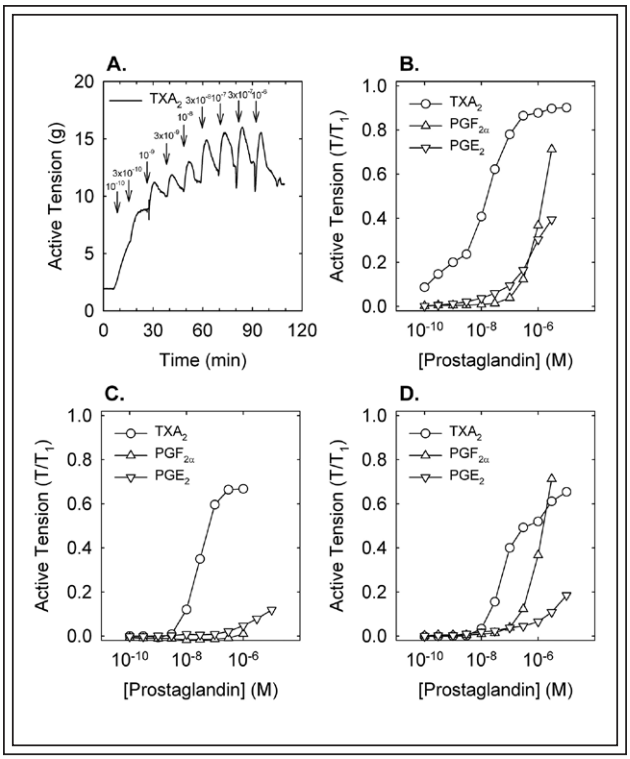

Figure 3. Cumulative prostaglandin dose-response curves produced in the presence of increasing molar concentrations of TXA_2 (open circles), $PGF_{2\alpha}$ (upward triangles), and PGE_2 (downward triangles). An example of a time-dependent force tracing from rabbit femoral artery is shown in panel "A." Panels B, C, and D are averaged dose-response data from rabbit femoral, carotid, and renal arteries, respectively obtained from 3 to 4 animals. This data serves as a control for the bioassay completed with strips of bladder from the same animal. T_o is defined as the maximum tension obtained with KCl contraction and is the value to which active tension (T) observed after drug administration was normalized (T/T_0). Treatment with DMSO control showed no effect (not represented here).

Cumulative concentration-response curves (Figure 3a, example tracing) for TXA_2, $PGF_{2\alpha}$, and PGE_2 were generated in femoral, Figure 3b, carotid, Figure 3c, and renal, Figure 3d, arteries. Femoral, carotid, and renal arteries contracted strongly to TXA_2, Figures 3b-3d. However, only femoral artery was highly sensitive to TXA_2, which produced a contraction nearly 10% of a KCl-induced contraction at 10^{-10} M (Figure 3b). Concentrations of 10^{-8} M and above were required to produce contractions of a comparable strength in carotid, Figure 3c, and renal, Figure 3d, arteries. All three artery types were relatively poorly sensitive to $PGF_{2\alpha}$ and PGE_2.

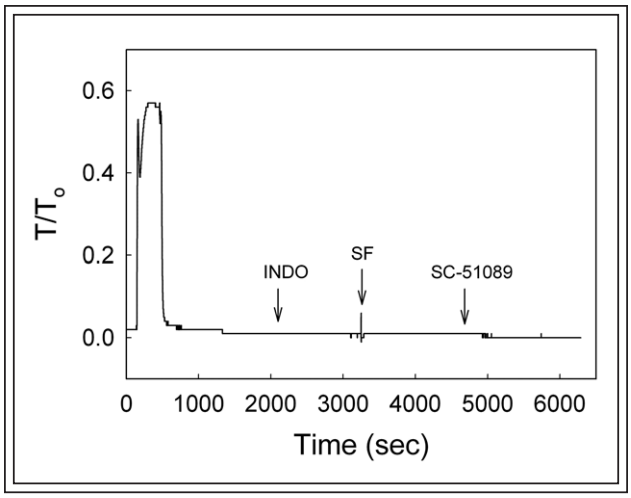

Figure 4. Representative bioassay in which rabbit femoral artery was initially contracted with KCl and then treated with 10^{-6}M indomethacin (Indo) to eliminate endogenous prostaglandin production. No change in tension was observed after medium was changed to superfusate (SF) obtained from a section of rabbit detrusor free of urothelium, and no change in tension was observed after treatment with 3µM SC-51089, the PGE_2 (EP1) antagonist.

Because of its high sensitivity to TXA_2 and low sensitivity and efficacy to PGE_2, femoral artery was chosen to serve as a negative control in the previously described four step bioassay. Because none of the artery types chosen for bioassay develop SRC, we did not expect indomethacin to affect basal tension, and this was indeed found to be the case (Figure 4, INDO). Addition of SF from a donor bladder consisting of detrusor muscle free of urothelium to a recipient femoral artery ring did not produce a contraction (Figure 4, SF) as did a recipient detrusor strip when exposed to SF, see Figure 1. As expected, addition of SC-51089 after addition of SF to femoral artery had no effect (Figure 4, SC-51089). Taken together, these results support the hypothesis that TXA_2 is not likely responsible for the generation of SRC because SF from detrusor did not increase tension in femoral artery.

PG production using EIA

To determine whether isolated strips of rabbit bladder free of urothelium produce prostaglandins, EIA analyses of total PG and PGE_2 production were performed. After 15 minutes incubation, there was a 2.5 ± 0.5-fold increase in PG production and a 4.8 ± 0.8-fold increase in PGE_2 production compared to respective 1 minute values (Figure 5a, n = 8 and Figure 5c, n = 6).

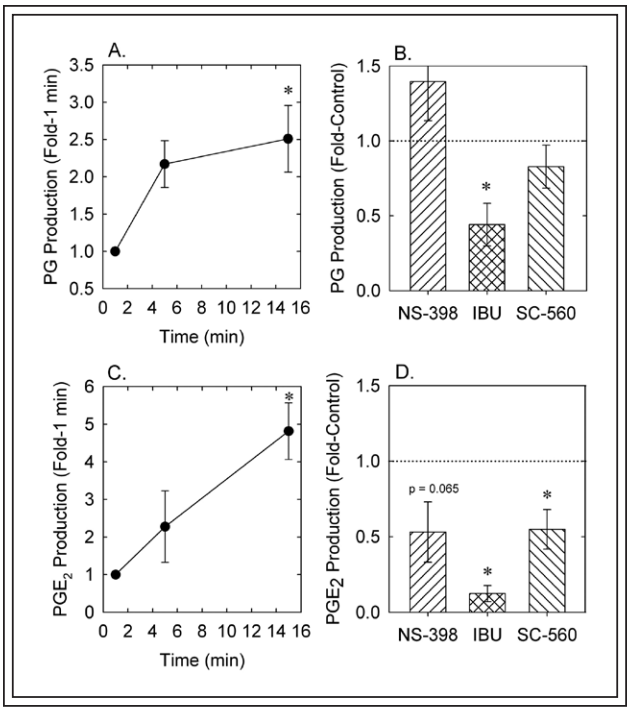

Figure 5. Results from the enzyme immunoassay (EIA) show time-dependent fold-change in production of prostaglandins (PGs; panel A, n = 8) and specifically, PGE_2 (panel C, n = 6). Reductions in PG (Panel B, n = 8) and PGE_2 (Panel D, n = 6) production after treatment with COX-2 inhibitor (10^{-7}M NS-398), ibuprofen (IBU, 30μM), or COX-1 inhibitor (10^{-7}M SC-560) were normalized to controls (data from Panel A and C at the 15 minute time point). *$p < 0.05$.

To determine the effects of a non-selective and of selective COX inhibitors on production of PGs and PGE_2, isolated strips of rabbit detrusor free of urothelium were incubated without (control) and with the non-selective COX inhibitor ibuprofen, the selective COX-1 inhibitor, SC-560, and the selective COX-2 inhibitor, NS-398. Media were processed using EIA to measure either PGs or PGE_2. Ibuprofen significantly diminished both PG and PGE_2 production. NS-398 and SC-560 at the low concentrations used did not reduce PG production, Figure 5b. However, SC-560 and NS-398 reduced the average value of PGE_2 production by 46% and 47%, respectively, Figure 5d, although the apparent reduction by NS-398 was not quite statistically significant ($p = 0.065$).

Discussion

This investigation confirms that PGE_2 is produced by rabbit bladder,[11] and demonstrates that PGE_2 production, at least in part, arises from the detrusor layer. Furthermore, a bioassay reveals that PGE_2 produced by detrusor can produce SRC. The physiologic role of SRC remains unknown despite its presence in mammalian species and man.[12] In addition, work by Drake and colleagues provide evidence that spontaneous activity exists in whole bladders and isolated bladder strips. These investigators used multipoint motion analysis to identify micro-contractions in unstimulated specimens.[13] Taken together, the data support our hypothesis that SRC is an intrinsic process arising from the detrusor layer and is possibly mediated by detrusor ICCs.

It has been speculated that the process of SRC might help in the accommodation of bladder size. Recent studies demonstrate that the passive length-tension curve for detrusor smooth muscle is not constant - but can "adjust" in response to a series of passive stretches. This process may underlie the ability of the detrusor to undergo large changes in length without compromising function.[10,14] In addition, in DSM, increased expression of the Gap Junction protein connexin-43 has been identified in animal models of detrusor overactivity.[15] In this "syncytium" model of detrusor overactivity, SRC might act to maintain or amplify Gap Junction-mediated contractile signaling.

Prostaglandins have been shown to regulate smooth muscle contractile activity in organs such as blood vessels, bowel, trachea, and uterus.[16] In the urinary bladder, the regulatory influence of prostaglandins has also been known for several decades.[17,18] However, the role of prostaglandins in the control of SRC is less well-studied. In the renal pelvis and proximal ureter, Davidson and Lang[19] demonstrated that SRC is suppressed by COX-1 and COX-2 antagonists. In smooth muscle from the corpora cavernosum of rabbit, Hashitani and collegues[20] demonstrated that spontaneous contractile activity was dependent on endogenous prostaglandin production. In urinary bladder, the exact cell type responsible for prostaglandin production and the mechanism through which prostaglandins mediate their effects is not entirely known.[21] However, studies on guinea pig bladder demonstrate that prostaglandins may be involved in the regulation of spontaneous contractile activity.[9] In these studies, intact bladder specimens displayed increased basal pressure as well as increased amplitude and frequency of autonomous (spontaneous) contractions when exposed to PGE_2. In addition, recent work using knockout mice and receptor pharmacology demonstrates that the excitatory effects of prostaglandins on detrusor function are likely receptor-mediated, possibly through the PGE_2 receptor, EP3.[22,23]

The current investigation demonstrates that PGE_2 production arises from the detrusor, possibly from interstitial cells of Cajal which express COX-1 and COX-2.[7] This study adds to our knowledge that the sub-urothelium (lamina propria) also is a source of PGE_2 as suggested recently by Nile and collegues.[24] In addition, studies showing potential PGE_2[8,25] production from the urothelium itself might highlight differing roles for PG arising from different bladder layers. The current work is limited by small sample sizes and the use of a single species (rabbit). In addition, arterial dose-response and bioassay data provide strong evidence that TXA_2 is not produced by the detrusor but, due to lack of a specific $PGF_{2\alpha}$ antagonist, contributory effects of $PGF_{2\alpha}$ on SRC was not ruled out.

Conclusions

Rabbit detrusor - produces PGE_2 which mediates the generation of SRC. Further study of SRC and the source of PGE_2 production may reveal rational targets for design of drugs to treat overactive bladder resistant to muscarinic receptor antagonists. □

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