PGE$_2$ increases release of substance P from renal sensory nerves by activating the cAMP-PKA transduction cascade

ULLA C. KOPP, MICHAEL Z. CICHA, AND LORI A. SMITH

Department of Internal Medicine, Department of Veterans Affairs Medical Center, Iowa City 52246; and University of Iowa College of Medicine, Iowa City, Iowa 52242

Received 26 November 2001; accepted in final form 31 January 2002

Kopp, Ulla C., Michael Z. Cicha, and Lori A. Smith. PGE$_2$ increases release of substance P from renal sensory nerves by activating the cAMP-PKA transduction cascade. Am J Physiol Regulatory Integrative Comp Physiol 282: R1618–R1627, 2002. First published February 28, 2002; 10.1152/ajpregu.00701.2001.—Increasing renal pelvic pressure increases afferent renal nerve activity (ARNA) by a PGE$_2$-mediated release of substance P (SP) from renal pelvic nerves. The role of cAMP activation in the PGE$_2$-mediated release of SP was studied by examining the effects of the adenylyl cyclase (AC) activator forskolin and AC inhibitor dideoxycadenosine (DDA). Forskolin enhanced the bradykinin-mediated release of SP from an isolated rat renal pelvic wall preparation, from 7.3 ± 1.3 to 15.6 ± 3.0 pg/min. PGE$_2$ at a subthreshold concentration for SP release mimicked the effects of forskolin. The EP$_2$ receptor agonist butaprost, 15 μM, and PGE$_2$, 0.14 μM, produced similar increases in SP release, from 5.8 ± 0.8 to 17.0 ± 2.3 pg/min and from 8.0 ± 1.3 to 21.6 ± 2.7 pg/min. DDA blocked the SP release produced by butaprost and PGE$_2$. The PGE$_2$-induced release of SP was also blocked by the PKA inhibitors PKI$_{14–22}$ and H-89. Studies in anesthetized rats showed that renal pelvic administration of butaprost, 10 μM, and PGE$_2$, 0.14 μM, resulted in similar ARNA responses, 1,520 ± 390 and 1,170 ± 270%·s (area under the curve of ARNA vs. time) that were blocked by DDA. Likewise, the ARNA response to increased renal pelvic pressure, 7,180 ± 710%·s, was blocked by DDA. In conclusion, PGE$_2$ activates the cAMP-PKA pathway leading to a release of SP and activation of renal pelvic mechanosensory nerve fibers. afferent nerves; EP$_2$ receptors; calcitonin gene-related peptide; kidney; forskolin; adenylyl cyclase; protein kinase A; mechanosensory nerves

PROSTAGLANDIN E$_2$ (PGE$_2$) is the major product of cyclooxygenase (COX)-induced metabolism of arachidonic acid in the kidney (2). PGE$_2$ plays a critical role in regulating renal hemodynamics and urinary sodium excretion (4, 6). Our previous studies indicated that PGE$_2$, in addition to its direct effect on tubular sodium reabsorption, may also modulate urinary sodium excretion by its effects on renal sensory nerves (28–31, 35, 36).

In the kidney, the majority of the sensory nerve fibers containing substance P and calcitonin gene-related peptide (CGRP) are located in the renal pelvic wall (27, 39, 55). Substance P plays a crucial role in the activation of renal mechanosensitive nerves (25, 28, 30, 34). Stretching the renal pelvic wall by increasing renal pelvic pressure leads to increased release and/or synthesis of bradykinin and activation of the phosphoinositide system and COX-2. Increased activation of COX-2 leads to increased PGE$_2$ synthesis in the renal pelvic wall (26, 28). PGE$_2$ increases the release of substance P from the renal pelvic sensory nerves by a Ca$^{2+}$-dependent mechanism that requires influx of Ca$^{2+}$ via N-type Ca$^{2+}$ channels (24). The increased release of substance P increases ARNA by activation of substance P receptors in the renal pelvic area (28, 33). Regarding the role of CGRP, our studies suggest that CGRP potentiates the effect of substance P by retarding the metabolism of substance P (13).

The increase in afferent renal nerve activity (ARNA) leads to a reflex decrease in efferent renal nerve activity and diuresis and natriuresis, a renorenal reflex response (32). The importance of this renorenal reflex in the renal control of water and sodium is suggested by the threshold of activation of the renal pelvic mechanosensory nerve fibers being less than 5 mmHg in rats fed a normal sodium diet (37). The sensitivity of the renal mechanosensory nerves is enhanced in rats fed a high-sodium diet and suppressed in rats fed a low-sodium diet (25). In this context, it is of interest that various mediators involved in the activation of renal sensory nerves, including renal COX-2 and PGE$_2$, are regulated by changes in dietary sodium intake (15, 38, 54). Enhanced activation of the renorenal reflex may contribute to the increased urinary sodium excretion during an excess sodium intake.

The important role of PGE$_2$ in the renorenal reflexes is shown by the marked impairment of the activation of renal mechanosensory nerves produced by COX inhibitors or an essential fatty acid-deficient diet. Renal pelvic administration of PGE$_2$ restores the responsiveness of the renal mechanosensory nerves toward normal values in the PGE$_2$-deficient animal models (31, 35).
PGE₂ mediate its action by increasing or decreasing cAMP activity or activating the phosphoinositide system via G protein-coupled receptors (4, 8, 41). At least four PGE₂ receptor isofoms have been cloned and are designated EP₁, EP₂, EP₃, and EP₄ (1, 8). Stimulation of EP₁ receptors activates phosphoinositidase C, whereas activation of EP₂ and EP₄ receptors stimulates adenylyl cyclase. Activation of EP₃ receptors may lead to increases or decreases in cAMP or activation of phosphoinositidase C (4, 8, 41). All four EP receptors are expressed in the kidney, albeit differentially along the nephron (19, 20). Although, the EP₂ receptor expression in the kidney has been shown to be much lower than that of the other EP receptors, it is of interest that the EP₃ receptor-deficient mouse develops hypertension when placed on a high-sodium diet (21). These findings may suggest that PGE₂-mediated activation of EP₂ receptors plays an important role in the renal control of sodium and water.

There is also extensive evidence for the four EP receptors being expressed on central sensory neurons (52). It is well established that PGE₂ activates the cAMP-protein kinase A (PKA) transduction cascade in cultured dorsal root ganglion (DRG) neurons with a resultant increase in the release of substance P and CGRP (10, 11, 17, 42, 47). A role for cAMP in the PGE₂-activation of peripheral sensory nerves has also been shown. PGE₂ was found to mimic the effects of cAMP analogs on cutaneous hyperalgesia (49) and abdominal sensory nerve activity (7, 14). Conversely, inhibition of adenylyl cyclase activity and PKA attenuated the PGE₂-mediated activation of ischemically sensitive abdominal visceral sensory nerves (14) and PGE₂-induced hyperalgesia (49), respectively.

However, little is known about the second messenger system involved in the PGE₂-evoked release of neuuropeptides from peripheral sensory nerves. Because of the extensive evidence for a role of the cAMP-PKA transduction cascade in PGE₂-mediated release of neuuropeptides from cultured DRG neurons, we examined whether known stimulators of cAMP activity increased renal pelvic release of substance P and whether inhibitors of the cAMP-PKA pathway decreased the PGE₂-mediated release of substance P from renal pelvic sensory nerves in an isolated renal pelvic wall preparation. Because these studies suggested a role for the cAMP-PKA pathway in the PGE₂-mediated release of substance P, we examined whether inhibition of cAMP activity modulated the activation of renal mechanosensory nerves in vivo.

METHODS

The study was performed on male Sprague-Dawley rats weighing 167–425 g (mean 296 ± 4 g) anesthetized with pentobarbital sodium, 0.2 mmol/kg ip.

In Vitro Studies

The procedures for stimulating the release of substance P from an isolated rat renal pelvic wall preparation were previously described in detail (24). In short, renal pelvices dissected from the kidneys were placed in wells containing 400 μl HEPES-indomethacin buffer maintained at 37°C. Indomethacin was included in the incubation buffer to minimize the influence of endogenous PGE₂ on substance P release (24) in all experimental protocols, except those in which the effects of bradykinin on substance P release were examined (see below). Each well contained the pelvic wall from one kidney. The same protocol was used to examine the effects of PGE₂ on renal pelvic release of CGRP.

The renal pelvic walls were allowed to equilibrate for 130 min. The incubation medium was gently aspirated every 10 min for the first 120 min and every 5 min thereafter. The medium was immediately replaced with fresh HEPES-indomethacin buffer to maintain PO₂ of the medium at 160–170 mmHg throughout the equilibration and experimental periods. All experiments, except those that examined the bradykinin-mediated release of substance P (see below), consisted of four 5-min control, one 5-min experimental, and four 5-min recovery periods. The aspirated incubation medium was placed in siliconized vials and stored at −80°C for later analysis of substance P or CGRP.

Effects of adenylyl cyclase activation on bradykinin-mediated release of substance P. Pilot experiments were performed to examine the subthreshold concentration of bradykinin for release of substance P. Pelvises from six rats were exposed to bradykinin at 19 or 38 μM during the experimental period, the ipsilateral and contralateral pelvices being exposed to different concentrations of bradykinin. Because these results showed a consistent increase in the release of substance P produced by bradykinin at 38 μM, from 9.8 ± 1.4 to 19.2 ± 2.8 pg/min (P < 0.01), but not by bradykinin at 19 μM, from 6.3 ± 1.4 to 10.1 ± 2.4 pg/min [not significant (NS)], the latter concentration was used in subsequent experiments.

Two groups were studied. In the first group (n = 8), the ipsilateral and contralateral pelvices were incubated in regular HEPES buffer for 20 min before being exposed to the adenylyl cyclase activator forskolin, 10 μM, and vehicle (0.1% DMSO), respectively, for 10 min. During the subsequent 5-min experimental period, bradykinin, 19 μM, was added to the ipsilateral and contralateral pelvices being incubated in HEPES-forskolin and HEPES-DMSO buffers, respectively. The experimental period was followed by a 20-min recovery period during which both pelvices were exposed to HEPES buffer only. In the second group (n = 11), the experimental protocol was identical, except PGE₂, 0.03 μM, at a subthreshold concentration for substance P release (25), was added to the incubation bath instead of forskolin.

Effects of PGE₂ and butaprost on renal pelvic release of substance P. Three groups were studied. The ipsilateral and contralateral renal pelvices were incubated in HEPES-indomethacin buffer. In the first two groups, the ipsilateral pelvis was exposed to PGE₂, 0.14 μM, and the contralateral pelvis to the EP₂ receptor agonist butaprost (8, 12), 10 (n = 6) or 15 μM (n = 10), during the experimental period.

In the third group (n = 7), the ipsilateral pelvis was incubated in HEPES-indomethacin buffer. The contralateral pelvis was incubated in HEPES-indomethacin buffer for 60 min and thereafter in HEPES-indomethacin buffer containing the adenylyl cyclase inhibitor dideoxyadenosine (DDA; 18), 10 μM. During the experimental period, both pelvices were exposed to butaprost, 15 μM, dissolved in the incubation medium.

Effects of adenylyl cyclase inhibition on PGE₂-mediated release of substance P and CGRP. Two groups were studied. In the first group (n = 12), both pelvices were incubated in HEPES-indomethacin buffer. During the experimental period, PGE₂, 0.14 μM, was added to the incubation bath of
both pelvies. The release of substance P and CGRP into the incubation baths was measured from the ipsilateral and contralateral pelvies, respectively. In the second group, the ipsilateral pelvis was incubated in HEPES-indomethacin buffer. The contralateral pelvis was incubated in HEPES-indomethacin buffer for 60 min and thereafter in HEPES-indomethacin buffer containing DDA, 10 μM. During the experimental period, the ipsilateral and contralateral pelvies in both groups were exposed to PGE₂, 0.14 μM, dissolved in the incubation medium. The release of substance P and CGRP into the incubation bath was measured from pelvies of 12 and 20 rats, respectively.

Effects of PKA inhibition on the PGE₂-mediated release of substance P. Two groups were studied. In the first group (n = 13), the ipsilateral pelvis was incubated in HEPES-indomethacin buffer. The contralateral pelvis was incubated in regular HEPES-indomethacin buffer until 30 min before the start of the experimental period and thereafter in HEPES-indomethacin buffer containing the cell membrane permeable specific PKA inhibitor myristoylated protein kinase inhibitor 14–22 amide (PKI14–22), 20 μM (16). During the experimental period, PGE₂, 0.14 μM, was added to the incubation bath of both pelvies. In the second group (n = 8), the experimental protocol was similar, except the PKA inhibitor H-89, 10 μM, was added to the bath of the contralateral pelvis instead of PKI14–22.

In Vivo Studies

After induction of anesthesia (see above), an intravenous infusion of pentobarbital sodium, 0.04 mmol·kg⁻¹·h⁻¹, at 50 μl/min into the femoral vein was started and continued throughout the course of the experiment. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA were previously described in detail (25–37). In short, left kidney was approached by a flank incision, a PE-10 catheter was placed in the right ureter for collection of urine, and a PE-60 catheter was placed in the left ureter with its tip in the pelvis. The left renal pelvis was perfused, via a PE-10 catheter placed inside the PE-60 catheter, throughout the experiment at 20 μl/min with vehicle or various renal perfusates administered in the different experimental protocols. ARNA was stimulated by increasing renal pelvic pressure by elevating a fluid-filled catheter above the level of the kidney or by administration of various agents into the renal pelvis at unchanged renal pelvic pressure, as described below. ARNA was recorded from the peripheral portion of the cut end of one renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-s intervals, the unit of measure being microvolts per second per 1 s. Postmortem renal nerve activity, which was assessed by crushing the decentralized renal nerve bundle peripheral to the recording electrode, was subtracted from all values of renal nerve activity. ARNA was expressed in percentage of its baseline value during the control period (25–37).

Experimental Protocols

Approximately 1.5 h elapsed after the end of surgery and the start of the experiment to allow the rat to stabilize as evidenced by 30 min of steady-state urine collections and ARNA recordings.

Effects of PGE₂ and butaprost on ARNA: role of cAMP. Two groups were studied. In the first group (n = 8), the experiment consisted of five parts. Each part consisted of a 10-min control, a 5-min experimental, and a 10-min recovery period. During the five experimental periods, 50 μl of 0.15 M NaCl, PGE₂, 0.14 μM, and butaprost, 4, 10, and 25 μM, were administered into the renal pelvis. The renal pelvis was not perfused during the control and recovery periods.

In the second group (n = 7), the experiment consisted of three parts separated by 10-min intervals. Each part consisted of two 10-min control, 5-min experimental, and 10-min recovery periods. PGE₂, 0.14 μM, and butaprost, 10 μM, were administered into the renal pelvis during the two experimental periods. The renal pelvis was perfused with vehicle (0.1% DMSO) during the first and third parts and DDA, 10 μM, during the second part of the experiment. The renal pelvic perfusates were switched immediately after the recovery periods.

Effects of adenylyl cyclase inhibition on the ARNA responses to increased renal pelvic pressure. The experiment consisted of three parts separated by a 10-min interval (n = 10). Each part consisted of a 10-min control, a 5-min experimental, and a 10-min recovery period. The left ureteral catheter was raised to increase renal pelvic pressure 15 mmHg during each experimental period. The renal pelvis was perfused with vehicle (DMSO) throughout the first and third parts of experiment and DDA, 10 μM, throughout the second part of the experiment. The renal pelvic perfusates were switched immediately after the recovery periods.

Drugs. Myristoylated PKI14–22 was acquired from Calbiochem (La Jolla, CA), substance P antibody (IHC 7451) and CGRP antibody (IHC 6006) from Penninsulas Laboratories (San Carlos, CA), and PGE₂ and butaprost from Cayman Chemicals (Ann Arbor, MI). All other agents were from Sigma Chemicals (St. Louis, MO). Indomethacin was dissolved together with Na₂CO₃ (2.1 weight ratio) in HEPES buffer. Butaprost, methyl acetate solution evaporated, forskolin, DDA, and H-89 were dissolved in DMSO and further diluted in the various incubation buffers (in vitro studies) or 0.15 M NaCl (in vivo studies), final DMSO concentration being 0.1%. Bradykinin, PGE₂, and PKI14–22 were dissolved in the various incubation buffers (in vitro studies) or 0.15 M NaCl (in vivo studies).

Analytic procedures. Right urinary sodium excretion measured during the experiment was expressed per gram kidney weight. Urinary sodium concentrations were determined with a flame photometer.

Substance P and CGRP in the renal pelvic effluent were measured by ELISA, as previously described in detail (24, 25, 27).

Statistical Analysis

In vitro, the release of substance P during the experimental period was compared with that during the control and recovery periods using Friedman two-way analysis of variance and shortcut analysis of variance. The Wilcoxon matched-pairs signed-rank test was used to compare the value of ipsilateral and contralateral renal pelvic release of substance P during the experimental period with the average of the control and recovery periods. In vivo, systemic hemodynamics and renal excretion were measured and averaged over each period. The ARNA responses to PGE₂, butaprost, and increased renal pelvic pressure were calculated as the area under the curve of ARNA vs. time (AUC), where ARNA was expressed as a percentage of its baseline value during the bracketing control and recovery period. Friedman two-way analysis of variance and shortcut analysis of variance were used to determine the effects of the various treatments on the ARNA responses within each rat. A significance level of 5% was chosen. Data in text and Figs. 1–6 are expressed as means ± SE (46, 50).
RESULTS

In Vitro Studies

Effects of adenylyl cyclase activation on bradykinin-mediated release of substance P. In the first set of experiments, we examined whether activation of adenylyl cyclase per se would increase baseline substance P release and/or enhance the release of substance P produced by bradykinin. In the absence of forskolin in the incubation bath, bradykinin, 19 \( \mu M \), had no effect on substance P release (Fig. 1). However, in the presence of forskolin, bradykinin produced a reversible release of substance P. Forskolin had no effect on basal substance P release. Likewise, adding PGE2 to the bath at a subthreshold concentration (0.03 \( \mu M \)) for substance P release (25) enhanced the bradykinin-mediated release of substance P (Fig. 1).

Effects of PGE2 and butaprost on renal pelvic release of substance P. Butaprost, known to increase cAMP activity via activation of its receptors coupled to cAMP via a Gs protein (1, 8), was added to the incubation bath to further substantiate a role for the cAMP-PKA pathway in the release of substance P from the renal pelvic nerves. Butaprost, 10 \( \mu M \), increased the release of substance P from 7.4 ± 1.3 to 12.7 ± 2.7 pg/min (\( P < 0.05 \)), which was less (\( P < 0.05 \)) than that produced by PGE2, from 10.1 ± 1.0 to 23.7 ± 2.6 pg/min (\( P < 0.01 \)). The increase in the release of substance P produced by butaprost, 15 \( \mu M \), was of a similar magnitude as that produced by PGE2 (Fig. 2).

To verify a role for cAMP in the butaprost-mediated increase in substance P release from the renal pelvic nerves, we compared the butaprost-induced substance P release in the presence and absence of DDA in the incubation bath. In the absence of DDA, butaprost failed to increase substance P release (Fig. 3).

Effects of adenylyl cyclase inhibition on PGE2-mediated release of substance P and CGRP. Substance P and CGRP are colocalized in the sensory nerve fibers in the renal pelvic wall (27). There is evidence to suggest that similar mechanisms control the release of the two neuropeptides in various sensory nerve fibers (40). We therefore tested the idea that PGE2 would increase the release of both substance P and CGRP and inhibition of adenylyl cyclase block the PGE2-mediated release of DDA, butaprost failed to increase substance P release (Fig. 3).

**Fig. 1.** Effects of bradykinin, 19 \( \mu M \), on the release of substance P from the isolated renal pelvic wall in the absence (vehicle) and presence of the adenylyl cyclase activator forskolin, 10 \( \mu M \) (A), and PGE2, 0.03 \( \mu M \) (B). Forskolin and PGE2, respectively, were present in the bath during minutes 20–35. **\( P < 0.01 \) vs. control and recovery periods; \( \ddagger P < 0.01 \) vs. effects of bradykinin on substance P release in the presence of vehicle.

**Fig. 2.** Effects of PGE2, 0.14 \( \mu M \), and the EP2 receptor agonist butaprost, 15 \( \mu M \), on the release of substance P from the isolated renal pelvic wall. **\( P < 0.01 \) vs. control and recovery periods.
the two neuropeptides. PGE₂ resulted in a reversible increase in the release of substance P and CGRP that was of a similar magnitude when expressed as percentage of baseline, the increases being 98 ± 29 and 90 ± 20%, respectively (Table 1). Further studies showed that in the presence of DDA in the incubation bath, the PGE₂-induced release of substance P and CGRP was significantly suppressed (Fig. 4 and Table 2).

Effects of PKA inhibition on the PGE₂-mediated release of substance P. To further establish a role for the cAMP-PKA transduction pathway in the PGE₂-mediated release of substance P from the renal pelvic sensory nerves, we examined the effects of two structurally different PKA inhibitors, myristoylated PKI₁₄₋₂₂ and H-89, on the release of substance P produced by PGE₂. In the absence of PKI₁₄₋₂₂ or H-89, PGE₂ produced a reversible increase in the release of substance P into the incubation bath (Fig. 4 and Table 3). However, in the presence of PKI₁₄₋₂₂ or H-89, the PGE₂-induced release of substance P was significantly reduced.

In Vivo Studies

Effects of butaprost and PGE₂ on ARNA: role of cAMP. To verify the importance of the cAMP-PKA pathway in renal sensory nerve activation, we examined whether butaprost and PGE₂ increased ipsilateral ARNA in vivo and, if so, whether inhibition of adenyl cyclase altered the increases in ARNA produced by butaprost and PGE₂. Renal pelvic administration of 50 μL vehicle (0.15 M NaCl) had no effect on ARNA. Renal pelvic administration of butaprost increased ARNA in a concentration-dependent fashion (Table 4). The ARNA response to butaprost, 10 μM, was of a similar magnitude and nature as that produced by PGE₂, 0.14 μM. There was an immediate increase in ARNA that gradually waned toward baseline values during the administration of either agent. Total duration of the ARNA responses to butaprost and PGE₂ was similar, being 117 ± 25 and 94 ± 19 s, respectively. Mean arterial pressure, 106 ± 2 mmHg; heart rate, 317 ± 9 beats/min, and basal ARNA, 1,770 ± 140 μV·s⁻¹·1 s⁻¹, did not change throughout the course of the experiment.

Subsequent experiments showed that renal pelvic perfusion with DDA produced a reversible inhibition of the ARNA responses to butaprost and PGE₂ (Fig. 5). Mean arterial pressure, 110 ± 3 mmHg; heart rate, 308 ± 7 beats/min; and basal ARNA, 1,560 ± 70 μV·s⁻¹·1 s⁻¹, remained unaltered throughout the course of the experiment.

Effects of adenyl cyclase inhibition on the ARNA response to increased renal pelvic pressure. Because the PGE₂-mediated release of substance P is a crucial mechanism in the activation of renal pelvic mechanoa sensory nerves (25, 28, 30, 34), we examined whether inhibition of adenyl cyclase reduced the ARNA response to increased renal pelvic pressure. Increasing renal pelvic pressure 15.1 ± 0.2 mmHg resulted in a significant increase in ARNA that was abolished by renal pelvic perfusion with DDA (Fig. 5). Switching the renal pelvic perfusate from DDA back to vehicle restored the ARNA response to increased renal pelvic pressure. The contralateral natriuretic responses to increased renal pelvic pressure paralleled the ARNA responses, the natriuretic responses being 13% from 1.0 to 1.4 μmol·min⁻¹·g⁻¹ (P < 0.01), 12 ± 7% from 1.7 to 1.9 μmol·min⁻¹·g⁻¹ (NS), and 35 ± 8% from 2.0 to 2.9 μmol·min⁻¹·g⁻¹ (P < 0.01) during vehicle, DDA, and vehicle perfusion, respectively. Mean arterial pressure, 111 ± 2 mmHg, and heart rate, 320 ± 9 beats/min, remained unaltered throughout the experiment. There was a slight decrease in basal ARNA during the course of the experiment, from 1,520 ± 110 to 1,350 ± 120 μV·s⁻¹·1 s⁻¹.

DISCUSSION

The results of our experiments in the isolated renal pelvic wall preparation show that PGE₂ mimicked the effects of forskolin to enhance bradykinin-mediated release of substance P. Butaprost and PGE₂ resulted in a vigorous increase in substance P release from the renal pelvic wall preparation that was abolished by renal pelvic perfusion with DDA (Fig. 5). The PKA inhibitors myristoylated PKI₁₄₋₂₂ or H-89 completely suppressed the increase in substance P release produced by butaprost in the presence of DDA.

### Table 1. Effects of PGE₂, 0.14 μM, on renal pelvic release of substance P and CGRP from an isolated renal pelvic wall preparation

<table>
<thead>
<tr>
<th>Substance P, pg/min</th>
<th>Control</th>
<th>PGE₂</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.1 ± 0.7</td>
<td>8.8 ± 2.3⁺</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>CGRP, pg/min</td>
<td>51 ± 7</td>
<td>110 ± 29⁺</td>
<td>57 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12). Control and recovery, average of four 5-min periods, respectively; ⁺P < 0.01 vs. control and recovery, CGRP, calcitonic gene-related peptide.
an increase in renal pelvic release of substance P that was blocked by inhibition of adenylyl cyclase. Likewise, inhibition of PKA blocked the PGE2-mediated renal pelvic release of substance P. Our in vivo studies show that renal pelvic administration of butaprost and PGE2 resulted in an increase in ipsilateral ARNA that was blocked by inhibition of adenylyl cyclase. Inhibition of adenylyl cyclase also blocked the ARNA response to increased renal pelvic pressure. Taken together, these studies suggest that activation of the cAMP-PKA transduction cascade contributes importantly to the PGE2-mediated release of substance P involved in the stimulation of the renal pelvic mechanosensory nerve fibers.

**Role of adenylyl cyclase in the PGE2-mediated release of substance P.** It is well established that PGE2 modulates the activity of ion channels that contribute to the resting cell membrane potential. PGE2 increases tetrodotoxin-resistant sodium current, inhibits voltage-gated potassium current, and increases Ca^{2+} in cultured DRG neurons. The PGE2-induced cell membrane depolarization is associated with a release of substance P and CGRP (52). There is substantial evidence for a role of the cAMP-PKA transduction cascade in these PGE2-induced events in DRGs (10, 11, 17, 42, 47). PGE2 has been shown to increase cAMP activity in renal pelvic tissue (23). It is likely that the PGE2-induced increase in cAMP activity, to some extent, is derived from nonneural renal pelvic tissue. However, studies in DRG neurons showing that PGE2 increases cAMP in central sensory nerves (17, 47, 48) suggest that the PGE2-induced increase in cAMP activity in renal pelvic tissue also stems from the sensory nerve fibers. We therefore hypothesized that PGE2 increases the release of substance P from renal pelvic sensory nerves via activation of the cAMP-PKA transduction cascade. The study was performed using the isolated renal pelvic wall preparation, which we previously established provided a valid model for studies of the mechanisms involved in the release of neuropeptides from the renal pelvic sensory nerves (24). We first examined whether activation of cAMP per se would alter the release of substance P from renal pelvic sensory nerves. Forskolin did not alter baseline substance P release but enhanced the release of substance P produced by bradykinin, which was added at a concentration that did not increase the release of substance P per se. Further studies showed that adding PGE2 to the bath at a subthreshold concentration for substance P release mimicked the effects of forskolin. These studies are in accordance with studies in DRGs showing that PGE2 mimics the effects of forskolin and cAMP analogs in enhancing the effects of bradykinin on substance P release (17, 47).

**Table 2. Effects of the adenylyl cyclase inhibitor, DDA, 10 μM, on PGE2-mediated release of CGRP from an isolated renal pelvic wall preparation**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>PGE2</th>
<th>Recovery</th>
<th>DDA</th>
<th>PGE2</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>179 ± 28</td>
<td>665 ± 126*</td>
<td>192 ± 28</td>
<td>162 ± 26</td>
<td>289 ± 48**</td>
</tr>
<tr>
<td>Recovery</td>
<td>165 ± 29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 20). Control and recovery, average of four 5-min periods, respectively; *P < 0.01 vs. control and recovery, †P < 0.01 vs. vehicle. DDA, dideoxyadenosine.
Butaprost activates EP receptors coupled to cAMP (1, 8). To further establish a role for cAMP activation in the mechanisms involved in the release of substance P, butaprost was added to the isolated renal pelvic wall. In the absence of DDA, butaprost produced a similar reversible increase in the release of substance P as PGE2. DDA prevented the butaprost-induced release of substance P. These data suggest that substance P can be released from the renal pelvic nerve fibers by activating cAMP via stimulation of EP receptors. Further studies showed that inhibition of adenylyl cyclase also blocked the PGE2-mediated release of substance P and CGRP.

Taken together, our studies using stimulators and inhibitors of cAMP activity suggest that PGE2 increases the release of substance P and CGRP from the renal pelvic nerves via activation of cAMP.

Role of PKA in the PGE2-mediated release of substance P. Numerous studies in cultured DRGs have established that PGE2-induced activation of the cAMP signaling pathway results in PKA-mediated phosphorylation of various proteins leading to modulation of the activity of ion channels with a resultant increased excitability of sensory neurons and neuropeptide release (11, 17, 47, 52). In the present study, the cell excitability of sensory neurons and neuropeptide re-signaling pathway results in PKA-mediated phosphorylation of renal pelvic sensory nerves.

ARNA produced by activation of EP receptors in the renal pelvic wall. Administering 10 μM butaprost into the renal pelvis resulted in an increase in ARNA that was of a similar magnitude and duration as that produced by 0.14 μM PGE2. Further studies showed that renal pelvic perfusion with DDA produced a reversible reduction of the ARNA responses to butaprost, PGE2, and increased renal pelvic pressure. Taken together these studies suggest that activation of the cAMP-PKA transduction cascade contributes to the PGE2-mediated activation of renal pelvic mechanosensory nerve fibers.

Source of various mediators involved in the activation of renal pelvic sensory nerves. Kinins are excreted in significant amount in the urine. In the kidney, the highest concentration of kinins is found in the terminal segments of the nephron and in the pelvis (3). The effects of increased renal pelvic pressure on the release of bradykinin have not been measured. However, studies in the urinary bladder showing that spontaneous contractions are associated with a release of bradykinin and substance P (44) may suggest that bradykinin is released during stretch of the renal pelvic wall. Our previous studies localized COX-2 activity and PGE2 synthesis to the renal pelvic wall (26, 30). Further evidence for PGE2 being synthesized in renal pelvic tissue stems from our pilot studies in the present study, which showed that the increase in the release of substance P produced by bradykinin, 38 μM, was associated with a release of PGE2 from 155 ± 32 to 480 ± 70 pg/min (P < 0.5, n = 6) from the renal pelvic tissue into the incubation bath. Whether COX-2 activity and PGE2 synthesis occur in the uroepithelial and/or muscular tissue surrounding the sensory nerves or in the sensory nerves per se cannot be deduced from these studies. Studies showing bradykinin-induced PGE2 synthesis in bladder uroepithelium (43) may suggest that PGE2 stimulating renal sensory nerves is, at least in part, derived from the renal pelvic uroepithelium. In addition to PGE2 in the renal pelvic tissue influencing the sensory nerves, the twofold higher concentration of bradykinin required to produce an increase in the release of substance P from renal pelvic nerves in vitro vs. in vivo (36) suggests that the renal pelvic sensory nerves...

Table 3. Effects of the PKA inhibitor H-89, 10 μM, on the PGE2-mediated release of substance P from an isolated renal pelvic wall preparation

<table>
<thead>
<tr>
<th>Vehicle</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P, pg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.4 ± 1.6</td>
<td>22.7 ± 2.7*</td>
<td>10.3 ± 1.4</td>
<td>11.9 ± 1.3</td>
<td>15.1 ± 1.7†‡</td>
<td>9.9 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). Control and recovery, average of four 5-min periods, respectively; *P < 0.01 vs. control and recovery; †P < 0.01 vs. vehicle; ‡P < 0.05 vs. recovery; n = 8.

Table 4. Effects of renal pelvic administration of PGE2 and butaprost on ipsilateral ARNA

<table>
<thead>
<tr>
<th>PGE2, μM</th>
<th>Butaprost, μM</th>
<th>ARNA, %·s (AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>4</td>
<td>1,175 ± 270*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>95 ± 35</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>1,520 ± 390*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,320 ± 410*</td>
</tr>
</tbody>
</table>

Values are means ± SE. ARNA, afferent renal nerve activity; AUC, area under the curve of ARNA response vs. time; *P < 0.01 vs. 0.
nerves may also be modulated by urinary and/or renal interstitial PGE₂. Of interest in this context are the findings of renal interstitial and urinary PGE₂ concentration being in the nanomolar range (9, 22, 28–31), i.e., within the range shown to increase renal pelvic release of substance P (24, 25, current study). Taken together, these studies suggest that PGE₂ from a non-neural source in the renal pelvic area may activate renal pelvic sensory nerve fibers. However, studies showing COX-2 activity and PGE₂ synthesis in central sensory nerves (51, 53) suggest that sensory neurons may be a source of PGE₂. These studies suggest the intriguing hypothesis that sensory nerve fibers may regulate the release of substance P by modulating their PG synthesis. The distribution of EP receptors to central sensory neurons (41, 52) may suggest that the EP receptors being activated by PGE₂ in the current study, are located on the peripheral sensory nerve terminals in the renal pelvic wall.

Does PGE₂ activate renal pelvic sensory nerves by stimulating EP₂ receptors? Although the focus of the current study was not to delineate the PGE₂ receptor isoform involved in the PGE₂-induced release of substance P, our findings suggest the involvement of EP₂ receptors in the neuropeptide release from the renal pelvic nerves. The lack of selective antagonists of EP receptors hampers the progress of identifying the various EP receptor isoforms involved in a physiological process. Butaprost is a relative selective EP₂ receptor agonist (1, 8, 12). In the current experiment, 10–15 μM butaprost-methyl ester increased the release of substance P and ARNA. Support for the hypothesis that the effects of butaprost are due to activation of EP₂ receptors in the current study may be derived from studies in rat and rabbit isolated nephron segments. Whereas 10 μM butaprost increased cAMP activity in rat descending thin limb of Henle’s loop in outer medulla, a nephron segment containing EP₂ receptors, 100 μM butaprost failed to increase cAMP activity in outer medullary collecting duct, a segment that contains EP₄ but not EP₂ receptors (20). Also in rabbit cortical collecting duct, a nephron segment that also contains EP₄ but not EP₂ receptors, luminal administration of 0.1 μM PGE₂ but not 10 μM butaprost hyperpolarized transepithelial voltage (45). Therefore, the analogous effects produced by butaprost and PGE₂ on renal pelvic release of substance P and ARNA in the current study may suggest that activation of EP₂ receptors contributes to PGE₂-mediated release of substance P from the renal pelvic nerves. Nevertheless, we cannot exclude the possibility that some of the effects of PGE₂ may also have been related to an effect on other cAMP-coupled EP receptors, including EP₃B, EP₃C, and EP₄ (41). A study by Southall and Vasko (48) using a combination of antisense oligonucleotides of EP₃C and EP₄ suggests a role for EP₃C and EP₄ in the PGE₂-induced increase in cAMP in DRG neurons. Further...
thermore, the expression of EP<sub>4</sub> mRNA in the epithelium of the renal pelvic wall (5) may support the notion that activation of EP<sub>4</sub> receptors contributes to PGE<sub>2</sub> stimulation of the renal sensory nerves. However, there is little information on the distribution of the other EP receptor subtypes in the renal pelvic wall.

In summary, the present study shows that PGE<sub>2</sub> mimics the effects of forskolin to enhance the bradykinin-mediated release of substance P. Butaprost, known to activate cAMP-coupled receptors, and PGE<sub>2</sub> result in increases in renal pelvic release of substance P and ipsilateral ARNA that are blocked by inhibition of adenyl cyclase. The PGE<sub>2</sub>-mediated release of substance P is also blocked by inhibitors of PKA. Thus agents that increase cAMP activity produced effects on renal pelvic release of substance P release and ARNA that are analogous to those produced by PGE<sub>2</sub>. A causal relationship between the activation of the cAMP-PKA transduction cascade and PGE<sub>2</sub>-induced release of substance P and activation of the renal pelvic nerves is established by the inhibitory effects produced by agents that block adenyl cyclase and PKA. Taken together, these studies suggest that activation of the cAMP-PKA transduction cascade is an important intracellular mechanism in the PGE<sub>2</sub>-elicited release of substance P and stimulation of the renal pelvic mechanosensory nerves (Fig. 6).

This work was supported by grants from the Department of Veterans Affairs, National Institutes of Health RO1-HL-66068, –Veterans Affairs, National Institutes of Health RO1-HL-66068–, and American Heart Association Grant-In-Aid 0150024N.

REFERENCES

29. Kopp UC, Farley DM, and Smith LA. Bradykinin-mediated activation of renal sensory neurons due to prostaglandin-depen-
CAMP MEDIATES PGE₂-INDUCED RELEASE OF SUBSTANCE P

R1627