Prostaglandins buffer ANG II-mediated increases in cytosolic calcium in preglomerular VSMC

KIT E. PURDY AND WILLIAM J. ARENDSHORST
Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7545

Purdy, Kit E., and William J. Arendshorst. Prostaglandins buffer ANG II-mediated increases in cytosolic calcium in preglomerular VSMC. Am. J. Physiol. 277 (Renal Physiol. 46): F850–F858, 1999.—In order to exert an appropriate biological effect, the action of the vasoconstrictive hormone angiotensin II (ANG II) is modulated by vasoactive factors such as prostaglandins PGE₂ and PG1₂. The present study investigates whether prostaglandins alter ANG II-mediated increases in cytosolic calcium concentration ([Ca²⁺]) in vascular smooth muscle cells (VSMC) isolated from rat renal preglomerular arterioles. [Ca²⁺]i was assessed using the calcium-sensitive dye fura 2 and a microscope-based photometer system. ANG II (10⁻⁷ M) caused a biphasic, time-dependent [Ca²⁺]i response: an initial peak increase from 52 ± 7 to 264 ± 25 nM, followed by a sustained plateau of 95 ± 9 nM in cultured VSMC. Coadministration of PGE₂ or PG1₂ or synthetic mimetics caused dose-dependent decreases in the peak [Ca²⁺]i response to ANG II, with attenuation of 40–50%. This degree of inhibition was even more pronounced in individual freshly isolated preglomerular VSMC. Increasing cAMP levels in cultured VSMC, by using either a cell-permeable analog or inhibiting phosphodiesterase activity, mirrored the antagonistic effects of prostaglandins on ANG II-stimulated increases in [Ca²⁺]i. Radioimmunoassays demonstrate that ANG II (10⁻⁷ M) stimulates production of PG1₂ and PGE₂; the stable prostacyclin metabolite 6-keto-PGF₁α was released in 10-fold greater concentrations than PGE₂. Indomethacin blockade of prostaglandin production potentiated both the peak (264 to 337 ± 26 nM) and sustained [Ca²⁺]i responses (95 to 181 ± 22 nM) to ANG II. When prostaglandin analogs were added during indomethacin treatment, the ANG II response was restored to the typical pattern. In conclusion, we demonstrate that modulation of intracellular calcium levels is one mechanism by which prostaglandins can buffer ANG II-mediated constriction in renal preglomerular VSMC. PG₁₂ is more potent than PGE₂ in this regard.

exogenous and endogenous prostaglandins was assessed using the calcium-sensitive dye fura 2 and a microscope-based fluorescence photometer system. In addition, the effect of increasing intracellular levels of cAMP independent of receptor activation on ANG II stimulation was examined. Radioimmunoassays (RIAs) were used to quantify prostaglandin release from these arteriolar cells before and after ANG II stimulation.

**METHODS**

Isolation of preglomerular resistance vessels. Experiments were performed on 200- to 300-g male Sprague-Dawley rats from our Chapel Hill breeding colony. To isolate VSMC from renal resistance vessels, we used a technique previously described by Zhu and Arendshorst (38) for the rat kidney. Sterile solutions and equipment were used throughout the procedure. Briefly, for each culture, three rats were anesthetized with pentobarbital sodium (60 mg/kg ip), and the abdominal aorta was cannulated below the renal arteries through a midline abdominal incision. The proximal aorta was compressed to halt blood flow, the left renal vein was cut, and the kidneys were perfused with ice-cold PBS (in mM: 17 K2HPO4, 3 Na2HPO4, 125 NaCl, and 5 MgCl2, pH 7.4) until renal venous effluent was blood free. Thereafter, the kidneys were perfused with approximately 5 ml of a magnetized iron oxide suspension (1% Fe3O4 in PBS), excised, and placed in fresh cold PBS. Thereafter, the isolation procedure was carried out on ice and in a sterile tissue culture hood, unless otherwise noted. After decapsulation, the cortex was dissected from the medulla. The cortical tissue was placed on a glass petri dish, gently minced with a razor blade for 3 min, and then transferred to a beaker with 5 ml cold PBS. Renal vessels containing iron oxide as well as surrounding connective tissue were removed from the solution with a magnet. The crude homogenate was then resuspended in PBS, passed through needles of decreasing size (22- and 23-gauge), and filtered through a 120-µm sieve. The microvessels were recovered from the top of the sieve and then purified once more by magnetic separation. The final preparation was digested with collagenase (8 mg/ml, type 1A; Worthington Biochemical, Lakewood, NJ) for 30 min with constant shaking at 37°C. After collagenase digestion, the tube was shaken vigorously to disperse the cells and iron oxide. The remaining solution consisted of isolated VSMC and short pieces of vessels (17).

Culture of VSMC. The method used to culture renal arteriolar VSMC has been described by Zhu and Arendshorst (38). Cells of the digested microvessels were collected by brief centrifugation and washed once with PBS to remove collagenase. Next, the cells were suspended in 36 ml culture medium (RPMI 1640, supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 0.6 mM l-glutamine, and 10% fetal calf serum (Hyclone Laboratories, Salt Lake City, UT)). The microvascular suspension was aliquoted into twelve 60-mm culture dishes and incubated at 37°C in 5% CO2-95% air at 98% humidity. The next day, the medium was changed and the cultures were subjected to serum-free medium 24 h before an experiment. Prior to the study, the VSMC were washed twice with physiological saline solution (PSS, containing in mM: 135 NaCl, 5 KCl, 1 CaCl2, 1 MgCl2, 5 d-glucose, and 10 HEPES, pH 7.4) and incubated in the dark at room temperature with 2 µM fura 2-AM for 60 min. After loading, the cells were washed three times with PSS and allowed to sit for 20 min. Immediately before testing, a coverslip was mounted in a plastic chamber, creating a well for drug addition directly over the center of the coverslip. The chamber was then centered in the field of a ×40 oil-immersion fluorescence objective of an inverted microscope (Olympus IX-70). Then cells were excited alternately with light of 340- and 380-nm wavelength from dual monochrometers of a Photon Technology International (PTI) dual-excitation wavelength Deltascan (model RMD). Fluorescence was detected by a photomultiplier after passing through a barrier filter (510 nm). Fluorescence signal intensity readings, [Ca2+]i, were calculated based on the ratio of 340/380 nm, according to the formula [Ca2+]i = ([Rmin] - R) / ([Rmax] - R) × (Smax × Smin) / Kd, described by Grynkiewicz et al. (13), using electronic calibration.

The effect of ANG II and its interactions with prostaglandin mimetics on renal VSMC were evaluated in terms of changes in [Ca2+]i. After a 50-s control period, ANG II (10-7 M) was added, and the response was measured for 200 s. Coadministration of stable synthetic analogs of PGE2 (15-(S)-15-methyl-PGE2, Cayman Chemical, Ann Arbor, MI) or PGI2 (iloprost; Berlex Laboratories, Cedar Knolls, NJ) and ANG II was used to determine the effects of exogenous prostaglandins on the calcium response to ANG II. These mimetics were tested in varying concentrations: 15-(S)-15-methyl-PGE2 (10-11 to 10-5 M) and iloprost (10-11 to 10-7 M), all dissolved in PSS. The actions of 15-(S)-15-methyl-PGE2 were confirmed by coadministration of native PGE2 (10-7 and 10-5 M). Conversely, the effect of endogenous prostaglandins was assessed by pretreating cells for 15 min with the cyclooxygenase inhibitor indomethacin (10-5 M). The effect of exogenous prostaglandins, without interference from endogenous compounds, was determined by coadministering the prostaglandin mimetics and ANG II on the background of indomethacin pretreatment. The effect of increasing intracellular levels of cAMP during ANG II stimulation was assessed by coadministration of two different compounds: 10-5 M dibutyl-cAMP, a cell-permeable cAMP analog, or 10-6 M milrinone, an inhibitor of cAMP phosphodiesterase PDE-3B (Sigma Chemical, St. Louis, MO). Each preparation was tested only once, to avoid possible receptor desensitization or tachyphylaxis.

Preliminary studies were conducted on freshly isolated cells from preglomerular arterioles according to methodology previously described (12, 17) to determine whether the PGI2 analog iloprost influenced ANG II–induced increases in [Ca2+]i. RIAs for PGE2 and 6-keto-PGF1α were used to determine endogenous prostaglandins. Cells were grown to confluence in 12-well plates and rendered quiescent by maintenance in serum-free medium 24 h before an experiment. On the day of the study, cells were washed twice with PSS and incubated at 25°C with various drugs. Cells were preincubated with indomethacin or vehicle for 15 min and then exposed to varying concentrations of ANG II for an additional 15 min. The culture supernatant was then removed and stored at −80°C until analysis. Cells were assayed for PGE2 and 6-keto-PGF1α (a stable metabolite of PGI2) using RIA kits purchased from PerSeptive Biosystems (Framingham, MA). Protein levels were determined using the Bradford method.
after digestion of cells with 1 M NaOH, as described previously (7).

Statistical analysis. Data are presented as means ± SE. Data sets were analyzed statistically with analysis of variance followed by post hoc testing according to Student-Newman-Keuls. P < 0.05 was considered statistically significant.

RESULTS

The stimulatory effect of ANG II was assessed in preglomerular VSMC using the calcium-sensitive dye fura 2. A representative tracing of the calcium-sensitive dye fura 2. A representative tracing of the ANG II effect is shown in Fig. 1. After recording basal control levels for 50 s, ANG II (10^{-7} M) was added to the chamber enclosing the coverslip of cultured VSMC. The raw data were recorded as photons emitted at 510 nm after excitation from dual monochrometers at 340 and 380 nm. These counts changed in an antiparallel fashion, as expected for typical behavior of fura-2 in response to a change in [Ca^{2+}]. The ratio of these counts is shown in Fig. 1B, and the calculated [Ca^{2+}] is shown in Fig. 1C. ANG II stimulation resulted in an immediate peak increase in [Ca^{2+}], lasting approximately 100 s, followed by a smaller, but sustained increase in [Ca^{2+}].

To determine the effect of exogenous prostaglandins on ANG II stimulation of [Ca^{2+}], biologically stable analogs of PGI2 (iloprost) and PGE2 [15-(S)-15-methyl-PGE2] were used to avoid the complications of rapid degradation of the native compounds. The prostaglandin mimetics alone had an insignificant effect on the baseline [Ca^{2+}]; 10^{-7} M iloprost caused an increase of 11 ± 4 nM (P > 0.4), and 10^{-5} M 15-(S)-15-methyl-PGE2 caused an increase of 15 ± 8 nM (P > 0.6). On average, ANG II alone caused [Ca^{2+}] to increase from a baseline of 52 ± 7 nM to a peak of 264 ± 25 nM and a sustained level of 95 ± 9 nM at 240 s (Fig. 2A). When given concurrently with ANG II at 50 s, iloprost (10^{-9} and 10^{-7} M) significantly reduced the immediate peak calcium response. Blockade by 10^{-7} M iloprost was evidenced by an ANG II-induced increase from a baseline of 59 ± 6 nM to a suppressed peak of 175 ± 19 nM (P < 0.001), with essentially no effect on the sustained phase. Figure 2B shows the change in [Ca^{2+}] calculated from the difference between the baseline readings and the immediate peak response. Iloprost dose-dependently attenuated the calcium response to ANG II; the highest concentration of iloprost tested (10^{-7} M) blocked ANG II stimulation by 50%. We did not test iloprost at higher concentrations because of limitations in the amount of the drug available.

15-(S)-15-methyl-PGE2 was less potent than iloprost when given simultaneously with ANG II at 50 s (Fig. 3A), although the qualitative effects on the ANG II calcium response were similar to those observed with iloprost. At 10^{-7} M, 15-(S)-15-methyl-PGE2 did not significantly affect the peak response to ANG II. However, this PGE2 analog at 10^{-5} M effectively attenuated the peak ANG II response to 191 ± 18 nM, a 42% change (P < 0.01). 15-(S)-15-methyl-PGE2 also decreased the peak response to ANG II in a dose-dependent fashion while having little effect on the flat plateau phase of the calcium response (Fig. 3B). To ensure that the PGE2 analog retains the specificity and potency of the native compound, the above experiments were repeated using native PGE2. The results obtained were almost identical to those using the analog. At 10^{-7} M, native PGE2 did not significantly affect the peak response to ANG II, whereas 10^{-5} M PGE2 attenuated the peak response to 176 ± 12 nM (P < 0.005). Thus, both native and synthetic PGE2 blunt the increase in [Ca^{2+}] elicited by ANG II.

Preliminary studies were conducted to verify that iloprost would attenuate the calcium response to ANG II in freshly isolated preglomerular arteriolar VSMC in a manner similar to that observed in the cultured preglomerular VSMC. In the individual fresh cells, ANG II (10^{-7} M) caused an immediate peak increase in
Ca\textsuperscript{2+} from a baseline of 111 ± 6 to 202 ± 22 nM (n = 7). Iloprost (10\textsuperscript{-7} M) attenuated this response by 83% (P < 0.01, n = 7).

Because prostaglandins are thought to exert their effects on VSMC via adenylate cyclase stimulation, the effect of increasing intracellular levels of cAMP was assessed during ANG II stimulation (Fig. 4). Two different approaches were used to increase cytosolic cAMP: 1) 10\textsuperscript{-5} M dibutyryl-cAMP, a cell-permeable cAMP analog, and 2) 10\textsuperscript{-6} M milrinone, an inhibitor of cAMP phosphodiesterase PDE-3B. This enzyme has been shown to be the primary PDE present in freshly isolated renal preglomerular VSMC (29). Coadministration of dibutyryl-cAMP with ANG II resulted in a significant attenuation of the peak [Ca\textsuperscript{2+}] response, from a baseline of 53 ± 4 to a peak of 177 ± 19 nM (P < 0.001). Similar results were observed with coadministration of milrinone, increasing from a similar baseline to a peak of 180 ± 9 nM (P < 0.001). Both maneuvers reduced the peak response to ANG II by ~50%. These results are not statistically different (P > 0.5) from those obtained by maximum attenuation with the exogenous prostaglandin analogs noted earlier.

To determine whether renal preglomerular VMSC, in the absence of endothelial cells, could produce endogenous prostaglandins in response to ANG II, we measured release of PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1a} (a stable metabolite of PGI\textsubscript{2}) into the cell culture supernatant using RIAs. In the absence of ANG II, VSMC produced 40 ± 10 pg 6-keto-PGF\textsubscript{1a} / 100 µg protein / 15 min (Fig. 5A). We could not detect basal release of PGE\textsubscript{2} above the lower limit of detection of the assay (10 pg / 100 µg protein / 15 min) (Fig. 5B). However, the addition of increasing concentrations of ANG II (10\textsuperscript{-11} to 10\textsuperscript{-7} M) stimulated the cells dose dependently to increase the synthesis of both prostaglandins. It is clear that 6-keto-PGF\textsubscript{1a} was produced at much greater amounts than PGE\textsubscript{2} during both basal conditions and in response to ANG II challenge. At 10\textsuperscript{-9} M ANG II, cells released 92 ± 13 pg 6-keto-PGF\textsubscript{1a} / 100 µg pro-

Fig. 2. Iloprost, a stable analog of PGI\textsubscript{2}, blunts the ANG II effect, reducing the peak calcium response. A: average responses in [Ca\textsuperscript{2+}] to stimulation at 50 s with 10\textsuperscript{-7} M ANG II alone (control) and varying concentrations of iloprost (10\textsuperscript{-9} and 10\textsuperscript{-7} M). B: changes in [Ca\textsuperscript{2+}] from baseline to peak values vary dose dependently with iloprost concentration (*P < 0.01). Values are means ± SE for 12 preparations in each group.

Fig. 3. [Ca\textsuperscript{2+}] in response to ANG II during treatment with 15-(S)-15-methyl-PGE\textsubscript{2}, a stable analog of PGE\textsubscript{2}. 15-(S)-15-methyl-PGE\textsubscript{2} blunts the peak calcium response. A: average responses of [Ca\textsuperscript{2+}] to stimulation at 50 s with 10\textsuperscript{-7} M ANG II alone (control) and combined with two concentrations of 15-(S)-15-methyl-PGE\textsubscript{2} (10\textsuperscript{-9} and 10\textsuperscript{-7} M). B: changes in [Ca\textsuperscript{2+}] from baseline to peak values vary dose dependently with 15-(S)-15-methyl PGE\textsubscript{2} concentration (*P < 0.01). Values are means ± SE for 12 preparations in each group.
tein$^{-1}·15$ min$^{-1}$ ($P < 0.01$), whereas PGE$_2$ production remained at nondetectable levels. At $10^{-7}$ M ANG II, the concentration used in fura 2 studies, both prostaglandins were released in significantly greater amounts than baseline levels: $459 \pm 40$ pg·100 µg protein$^{-1}·15$ min$^{-1}$ for 6-keto-PGF$_{1alpha}$ ($P < 0.001$) and approximately 10 times less, $42 \pm 8$ pg·100 µg protein$^{-1}·15$ min$^{-1}$, for PGE$_2$ ($P < 0.01$). To ensure that the RIA was specific for cyclooxygenase products, the cells were pretreated with the cyclooxygenase blocker indomethacin for 15 min and then subjected to the same treatment. Indomethacin decreased release of both prostaglandins to undetectable levels, even at the highest concentration of ANG II.

To determine the effect of endogenous prostaglandins on the calcium response to ANG II, cultured VSMC were pretreated with indomethacin for 15 min before calcium determinations. Cyclooxygenase blockade resulted in elevated baseline levels of [Ca$^{2+}$]$_i$ from $52 \pm 7$ to $85 \pm 7$ nM (Fig. 6), indicating that prostaglandins are produced and exert effects under basal conditions. Moreover, indomethacin potentiated ANG II effects on [Ca$^{2+}$]$_i$, both in terms of the immediate peak (from $264 \pm 25$ to $337 \pm 26$ nM) and the sustained phase (from $95 \pm 9$ to $181 \pm 22$ nM). These observations indicate that prostanoids synthesized by the cyclooxygenase pathway blunt both the immediate and sustained phases of the calcium response to ANG II stimulation.

Other experiments were conducted to determine the effects of exogenous prostanoids during cyclooxygenase inhibition. In these studies, VSMC were pretreated with indomethacin for 15 min, and then exogenous prostaglandins were administered at the maximal concentrations used previously to block ANG II effects. When basal production of prostaglandins was blocked, all groups exhibited an elevated baseline [Ca$^{2+}$]$_i$ of $80 \pm 5$ nM, an increase of $25$ nM above that in untreated VSMC (Fig. 7A). When added concurrently with ANG II in the presence of indomethacin, iloprost ($10^{-7}$ M) caused a small decrease in the peak response from $337 \pm 26$ to $296 \pm 46$ nM, which was not statistically significant ($P > 0.4$), and a larger decrease in the sustained response, from $181 \pm 22$ nM to $98 \pm 10$ nM ($P < 0.005$). Thus, iloprost treatment during indomethacin...
PGE\(_2\) and PGI\(_2\) can significantly attenuate the ANG II stimulation in a dose-dependent fashion. To our knowledge, only one other study has specifically examined the effects of an arachidonic acid derivative on ANG II-mediated changes in intracellular calcium, and it dealt with lipoxygenase metabolites (28).

The physiological importance of the modulatory effect of prostaglandins on the microvasculature of the kidney is well known. For instance, patients with elevated ANG II activity experience acute renal failure when given nonsteroidal anti-inflammatory drugs that block cyclooxygenase, because the unopposed ANG II-mediated vasoconstriction severely reduces renal blood flow and glomerular filtration rate (25, 27). We (8) and others (18) have shown that a defect in this buffering system is associated with the development of hypertension in genetically susceptible rats.

Our observations indicate that several mechanisms are involved in receptor-mediated increases in [Ca\(^{2+}\)]\(_i\), and that prostaglandins appear to interact with them differently. ANG II receptor stimulation in the preglomerular VSMC results in a biphasic response consisting of an immediate peak increase in [Ca\(^{2+}\)]\(_i\), followed by a sustained increase of lesser magnitude. Prostaglandin analogs given in the presence of endogenous prostaglandins predominantly attenuate the peak (Figs. 2A and 3A), whereas blockade of endogenous prostanooids potentiates both peak and plateau portions of the response (Fig. 6). This difference can be explained if distinct mechanisms are responsible for the increases in [Ca\(^{2+}\)]\(_i\) observed during peak and sustained phases. Exogenous prostaglandins can act additively with endogenous compounds to further suppress the peak response to ANG II. However, endogenous prostaglandins appear to be saturating the mechanism responsible for the sustained phase so that no additional buffering is observed when exogenous mimetics are added. This view is supported by evidence indicating that both calcium mobilization from intracellular stores and calcium entry participate in ANG II-mediated constriction in renal resistance vessels (26) and, more specifically, preglomerular VSMC (10, 16).

It has been generally thought that prostaglandins are primarily if not exclusively synthesized by endothelial cells, with subsequent diffusion to adjacent VSMC to exert their effect (22, 35). However, several reports utilizing fresh cell preparations in various vascular beds (e.g., pulmonary, coronary, and aortic vessels) suggest a synthetic role for VSMC in addition to the
endothelium (4, 34, 39). A recent immunological study reports that cyclooxygenase-1 is expressed in preglomerular vessels of human kidneys (22). A previous study of cultured rabbit renal preglomerular VSMC reported synthesis of the prostacyclin (PGI₂) metabolite 6-keto-PGF₁α (2 ng·mg protein⁻¹·15 min⁻¹) and PGE₂ (11 ng·mg⁻¹·15 min⁻¹) in the basal state (9). These results, however, contrast with our data indicating that unstimulated cultured VSMC produce 6-keto-PGF₁α (0.4 ng·mg⁻¹·15 min⁻¹), whereas PGE₂ production is below the detection limits of the assay. A novel observation in the present report is that ANG II stimulation significantly increases production of both PGI₂ (to 5 ng·mg⁻¹·15 min⁻¹) and PGE₂ (to 0.4 ng·mg⁻¹·15 min⁻¹) in renal preglomerular VSMC. This is consistent with earlier work showing that a freshly isolated membrane preparation from rabbit preglomerular microvessels with an intact endothelium produce three times as much prostacyclin metabolite 6-keto-PGF₁α as PGE₂ (20). Previous studies on cultured VSMC from nonrenal vessels indicate greater production of 6-keto-PGF₁α compared with PGE₂ during ANG II stimulation (5, 14). Thus, our data, together with those of other investigators, suggest that PGI₂ is produced in greater abundance than PGE₂ in renal preglomerular VSMC. One possible caveat is that RIA antibodies may bind to metabolites from other arachidonic acid pathways. However, because indomethacin completely abolishes release of both prostanoid compounds as measured by RIA, it is reasonable to conclude that the antibodies are specific to cyclooxygenase products.

To minimize the potential influence of basal prostanoid activity in some of our calcium studies, indomethacin treatment was employed and exogenous prostaglandins were coadministered with ANG II. These experiments demonstrate that the indomethacin effect is reversed by coadministration of either prostanoid. This confirms that cyclooxygenase blockade with indomethacin is specific, in agreement with the elimination of prostaglandin release by indomethacin treatment in the RIA. These data argue against the possibility that the observed effects are due to indomethacin rerouting arachidonic acid metabolism to alternative enzymatic pathways, thereby increasing the levels of an eicosanoid vasoconstrictor rather than decreasing levels of cyclooxygenase-derived vasodilators. Furthermore, these results exclude the involvement of the vasoconstrictor thromboxane, another cyclooxygenase product. Previous studies have shown that thromboxane analogs can increase [Ca²⁺] and constrict aortic VSMC (11, 33).

The effects of endogenous prostaglandins and PGE₂ and PGI₂ analogs on [Ca²⁺] during ANG II stimulation can be compared by subtracting the experimental from control curves obtained in our experiments (Figs. 6 and 7). This analysis, shown in Fig. 8, effectively cancels the effect of ANG II on [Ca²⁺], showing the change in [Ca²⁺] specifically elicited by prostaglandins. The curve labeled endogenous prostaglandins (Fig. 8A) shows the difference between the data in Fig. 6 for ANG II alone and ANG II with indomethacin. The remaining curves (Fig. 8, B and C) represent the subtraction of exogenous prostaglandin treatment from ANG II alone, both during cyclooxygenase blockade (Fig. 7, A and B). Endogenous prostaglandins lower the baseline [Ca²⁺] by ~40 nM (Fig. 8A). The baseline change is approximately zero in Fig. 8, B and C, however, because both curves within a group were treated with indomethacin, and thus the two curves showed approximately the same baseline values for [Ca²⁺]. Both prostaglandin analogs effectively restore the sustained [Ca²⁺] response observed with endogenous prostaglandin action. With all
interactions, \([\text{Ca}^{2+}]\), during the sustained phase is reduced by approximately 80 nM from basal levels. However, the kinetics of the response differ. The effect of iloprost is similar to that of endogenous prostaglandins, although a bit slower; it takes about 50 s longer for iloprost to exert a maximum effect in reducing \([\text{Ca}^{2+}]\). 15-(S)-15-methyl-PGE_{2} actually shows some vasoconstrictor-like activity by initially increasing \([\text{Ca}^{2+}]\) and then reducing it to an apparent maximum of 80 nM. This phenomena could be explained by the expression of multiple PGE_{2} receptor subtypes in the renal preglomerular VSMC. Some subtypes (EP_{1}, EP_{3}) are known to mediate vasoconstriction, whereas others (EP_{2}, EP_{4}) are thought to elicit vasodilation (32). Only one receptor subtype has been identified for PGI_{2}, the IP receptor. The calcium data discussed above, together with greater release of 6-keto-PGF_{1α} and greater potency of iloprost in the earlier calcium experiments, suggest that prostacyclin is the primary vasoconstrictor cyclooxygenase product involved in the ANG II response in renal preglomerular VSMC.

The mechanisms by which prostaglandins affect \([\text{Ca}^{2+}]\) are incompletely understood. PGE_{2} and PGI_{2} are known to activate adenylate cyclase and to increase intracellular cAMP levels in several different vascular and epithelial cells, including renal preglomerular VSMC preparations (9, 27). In the current preparation, we have shown that increasing intracellular cAMP levels attenuates the peak \([\text{Ca}^{2+}]\) response during ANG II stimulation (Fig. 4). The magnitude of this effect is similar to that observed with addition of exogenous prostaglandins (Fig. 2). These results are consistent with the hypothesis that prostaglandins can exert their vasoconstrictor actions via adenylate cyclase stimulation.

cAMP is thought to activate PKA to carry out the intracellular effects of prostaglandins. However, the relationship between increased PKA activity and changes in \([\text{Ca}^{2+}]\) remains imprecise. One view is that PKA phosphorylates and thereby increases the activity of calcium ATPases that remove calcium from the cytoplasm. Accumulating evidence suggests that the primary effect of PKA is on a calcium-ATPase in the sarcoplasmic reticulum to increase calcium reuptake (23). PKA also has been reported to phosphorylate the IP_{3} receptor, thereby reducing its efficiency to release calcium from intracellular stores (31). It is also possible that PKA can affect calcium entry and/or the sensitivity of the contractile apparatus. Indirect support for this view comes from studies in which iloprost inhibits KCl-induced contraction to a greater degree than it reduces \([\text{Ca}^{2+}]\) (24). Several reports indicate that prostaglandins can act through PKA to modulate potassium channel activity (10, 30).

In conclusion, both PGI_{2} and PGE_{2} effectively attenuate the stimulation of \([\text{Ca}^{2+}]\) by ANG II. This was the case for cultured VSMC from rat preglomerular arterioles before and during inhibition of cyclooxygenase and production of endogenous prostanooids. Prostacyclin also blocks ANG II-induced stimulation of \([\text{Ca}^{2+}]\) in individual freshly isolated renal arterial VSMC. Our calcium results demonstrate that the buffering effect of prostacyclin is closely mimicked by endogenous prostanooids. The counteracting effects of endogenous cyclooxygenase products as well as PGI_{2} and PGE_{2} analogs are most likely mediated by cAMP. Cell-permeable cAMP and increases in endogenous cAMP associated with phosphodiesterase inhibition by milrinone produced effects similar to those of the prostanooids analogs. The RIA data indicate that cultured preglomerular VSMC release more PGI_{2} than PGE_{2} in response to ANG II. Thus, it is tempting to speculate that prostaglandins may act in an autocrine/paracrine manner in the afferent arteriole. This work demonstrates that modulation of \([\text{Ca}^{2+}]\), is one mechanism by which prostaglandins may attenuate ANG II-mediated constriction in the renal microcirculation.

We are grateful to Dr. Susan K. Fellner for conducting the preliminary studies on freshly isolated renal arterial VSMC. Iloprost was a generous gift of Berlex Laboratories.

We appreciate the assistance of J. Vile Vorobiov on prostaglandin assays performed at a core facility supported by National Institutes of Health Grant P30-DK-34987. This research was supported by National Heart, Lung, and Blood Institute Grant HL-02334. K. E. Purdy was supported by a Howard Hughes Predoctoral Fellowship. Accepted for reprint requests and other correspondence: W. J. Arendshorst, Department of Cell and Molecular Physiology, Room 152, Medical Sciences Research Building, CB 7545, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7545 (E-mail: arends@med.unc.edu).

Received 21 December 1998; accepted in final form 9 July 1999.

REFERENCES


