Protein Kinase C and Calcium Channel Activation as Determinants of Renal Vasoconstriction by Angiotensin II and Endothelin

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The mechanisms mediating renal microvascular constriction induced by angiotensin II (Ang II) and endothelin (ET) have not been fully established. In the present study, we have determined the effects of isradipine, a dihydropyridine calcium antagonist, on Ang II– or ET-induced constriction of afferent arterioles (AAs) and efferent arterioles (EAs) using the isolated perfused hydronephrotic kidney. Ang II (0.3 nmol/L) and ET (0.3 nmol/L) constricted AAs by 36±2% and 29±3%, respectively. Isradipine reversed AA constriction induced by both peptides. However, Ang II–induced AA constriction was more sensitive to isradipine than ET-induced constriction (half-maximal inhibitory concentration [IC50], 1.2±0.2 nmol/L [n=12] versus 170±65 nmol/L [n=19]; P<.01). The sensitivity of Ang II–induced AA constriction to isradipine was identical to that of KCl-induced AA constriction (IC50, 4.2±0.9 nmol/L; n=12). Pretreatment with staurosporine (50 nmol/L), a protein kinase C inhibitor, enhanced the sensitivity of ET-induced AA constriction to isradipine (4.3±1.7 nmol/L, n=14), rendering it identical to that of KCl-induced AA constriction. Ang II and ET decreased EA diameter by 26±2% (n=12) and 12±2% (n=8), respectively. In contrast to AA constriction, EA constriction induced by both peptides was relatively refractory to isradipine. Our data indicate that, whereas Ang II–induced AA constriction is primarily mediated by the activation of voltage-dependent calcium channels, activation of protein kinase C in addition to voltage-dependent calcium channels is involved in ET-induced AA constriction. Our results further suggest that EA constriction induced by Ang II and ET are relatively independent of the activation of voltage-dependent calcium channels. (Circ Res. 1993;73:743-750.)

KEY WORDS • protein kinase C • Ca2+ channels • renal vasoconstriction

The effects of vasoactive peptides on the renal vasculature have been extensively studied by many investigators.1-4 An extensive body of evidence indicates that angiotensin II (Ang II) is a potent renal vasoconstrictor. In addition to its physiological roles in cardiovascular homeostasis and renal hemodynamics,1 Ang II has been shown to play an important role in mediating pathophysiological renal vasoconstriction in a wide array of disorders, including heart failure, hypertension, and progressive renal disease.5-7

Recently, Yanagisawa et al8 have isolated a novel constrictor peptide, endothelin (ET). This peptide consists of 21 amino acids and is synthesized by endothelial cells.8 ET is considered to be the most potent vasoconstrictor known and has been postulated to play a pathogenic role in mediating renal vasoconstriction induced by either ischemia or cyclosporine.2

On the basis of the above considerations, elucidation of the mechanisms whereby Ang II and ET induce renal vasoconstriction would be of great interest and would provide a theoretical framework for developing strategies for the management of diverse renal functional disorders. We9 and other investigators10,11 have previously demonstrated that renal vasoconstriction induced by either Ang II or ET involves the activation of voltage-dependent calcium channels. However, the relative contribution of this mechanism to vasoconstrictor actions of either Ang II or ET on the renal microvasculature has not been heretofore established. Furthermore, we have reported that Ang II–induced renal vasoconstriction was reversed by the removal of this peptide, whereas ET–induced renal vasoconstriction was sustained even after the removal of the peptide.9 These observations suggest that, in addition to the mechanisms mediating Ang II–induced renal vasoconstriction, other mechanisms that markedly prolong the vasoconstrictor actions of ET are involved in renal vasoconstriction induced by this peptide.

In the present study, we have examined the effects of isradipine, a potent calcium antagonist that selectively inhibits calcium current through voltage-dependent calcium channels,12,13 on Ang II– or ET–induced renal microvascular constriction. In addition, because ET has been demonstrated to activate protein kinase C in vascular smooth muscle,14 we have also investigated the effects of isradipine on ET–induced renal microvascular constriction in the presence of staurosporine, a protein
kinase C inhibitor.\textsuperscript{15} The present data provide evidence indicating that, whereas Ang II–induced afferent arteriolar constriction is primarily mediated by voltage-dependent calcium channels, the activation of protein kinase C in addition to voltage-dependent calcium channels is involved in ET-induced afferent arteriolar constriction. Our results further suggest that efferent arteriolar constriction induced by both Ang II and ET is relatively independent of voltage-dependent calcium channels.

**Materials and Methods**

**Animal Preparation**

Isolated perfused hydronephrotic kidneys were used to examine the role of protein kinase C and voltage-dependent calcium channels in mediating renal microvascular constriction induced by either Ang II or ET. Twenty-five Sprague-Dawley rats (Charles River Laboratories, Inc, Wilmington, Mass) were used for the induction of hydronephrosis. The right ureter of each animal was exposed by a small abdominal incision and ligated under methoxyflurane anesthesia (Metofane, Pittman-Moore, Mundelein, Ill). After 4 to 8 weeks, the hydronephrotic kidney was harvested for study. At this point, renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels.\textsuperscript{16}

**Perfusion of Hydronephrotic Kidneys**

For the perfusion of the hydronephrotic kidneys, the rats were anesthetized with methoxyflurane. The renal artery of the hydronephrotic kidney was cannulated by introducing the perfusion catheter through the mesenteric artery and across the aorta. Perfusion with warm oxygenated media (pH 7.4) was initiated in situ during this cannulation procedure to avoid ischemia of the perfused kidney. The hydronephrotic kidney was then excised and placed on the stage of an inverted microscope (model K, Nikon) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom.

The perfusion media consisted of Krebs-Ringer bicarbonate buffer containing 6.5 g/100 mL bovine serum albumin (Bovuminar, Intergen Co, Purchase, NY), 5 mmol/L d-glucose, and a complement of amino acids, as described previously.\textsuperscript{17} Perfusate was provided to the kidney at a constant pressure from a pressurized chamber. The chamber pressure was maintained by the inflow of warm hydrated gas at 95% O\textsubscript{2}–5% CO\textsubscript{2}, which exited through an adjustable back-pressure regulator (model 10BP, Fairchild Industrial Products Co, Winston-Salem, NC). Perfusion pressure was monitored at the level of the renal artery and maintained constant at 80 mm Hg throughout the experiments to avoid pressure-induced vasoconstriction.\textsuperscript{18} The effluent was returned to the pressurized chamber by two rolling pumps (Masterflex, Chicago, Ill).

Video images of renal microvessels were obtained by use of a video camera (model ITC-47, Ikegami, Tokyo, Japan) and recorded by a videocassette recorder (model nv-8950, Panasonic). Vessels were selected for study on the basis of adequate flow as estimated by the response to temporary (approximately 1 second) occlusion of the perfusion line. Vessels that exhibited a sluggish or blunted response were considered to be perfused inadequately and were excluded from the study. To determine vessel diameter, the video recording was transmitted to an IBM-AT computer equipped with a display board (model IVG-128, Datacube, Inc, Peabody, Mass). Vessel diameters were estimated with an automated program custom-designed to determine the mean distance between parallel edges. The renal microvessel diameters were measured during the plateau of the response. A segment of an afferent or efferent arteriole approximately 10 μm in length was scanned at 2- to 5-second intervals.

**Experimental Protocols**

In all experiments, kidneys were allowed to equilibrate at least for 30 minutes before basal measurements were obtained. Isolated perfused hydronephrotic kidneys were then divided into five groups. For the first group of kidneys (n=6), Ang II (0.3 nmol/L) was directly administered to the perfusate. Subsequently, increasing doses of isradipine (from 0.1 nmol/L to 1 μmol/L) were added.

In a series of preliminary studies, we characterized the temporal profile of renal microvascular constriction in response to the administration of Ang II (0.3 nmol/L). The vasoconstriction induced by Ang II persisted for a period of observation exceeding 2 hours.

In a second group of kidneys (n=5), the potassium concentration of the perfusate was iso-osmotically increased to 30 mEq/L. Increasing doses of isradipine (from 0.1 nmol/L to 1 μmol/L) were then added to the perfusate. Since we have previously demonstrated that membrane depolarization induced by 30 mEq/L potassium primarily constricts afferent arterioles,\textsuperscript{19} we have observed only afferent arteriolar responsiveness in this group.

In a third group of kidneys (n=7), ET (0.3 nmol/L) was added to the perfusate. Isradipine was then administered in a dose-dependent manner to achieve final concentrations of 0.1 nmol/L to 1 μmol/L. The doses of the vasoconstrictor stimuli (Ang II, KCl, and ET) were selected on the basis of the ability of each agonist to reduce renal perfusate flow by approximately 50%, as detailed previously.\textsuperscript{9,20}

To determine the contribution of protein kinase C to ET-induced constriction, kidneys in the fourth group of rats (n=6) were pretreated with staurosporin (50 nmol/L) at least for 30 minutes. Then ET (0.3 nmol/L) was directly administered to the media. Subsequently, increasing doses of isradipine (from 0.1 nmol/L to 1 μmol/L) were added.

The kidneys in the fifth group (n=4) were pretreated with staurosporin (50 nmol/L) for at least 30 minutes to assess the specificity of staurosporin. Then, the potassium concentration of the perfusate was increased iso-osmotically to 30 mEq/L, and changes in afferent arteriolar diameter were recorded. Subsequently, increasing doses of isradipine (from 0.1 nmol/L to 1 μmol/L) were added directly to the media.

Ang II and porcine ET I were obtained from Sigma Chemical Co, St Louis, Mo, and Peninsula Laboratories, Inc, Belmont, Calif, respectively. These peptides were dissolved in water and kept frozen until usage. Staurosporin was purchased from Calbiochem Corp, La Jolla, Calif, and dissolved in dimethyl sulfoxide.
Isradipine, a dihydropyridine calcium antagonist, was kindly provided by Sandoz Pharmaceuticals, East Hanover, NJ. The stock solution of isradipine was freshly prepared on the day of the experiment and dissolved in polyethylene glycol (Sigma). Sodium lighting and yellow filter were used throughout the experiments to avoid photodegradation.

Statistics

Data were first analyzed by analysis of variance. To compare the means among different groups, the Newman-Keuls test was used. A paired t test was applied for comparison between the means within a single group. To compare the half-maximal inhibitory concentrations, data were converted to natural logarithms to render the variance identical. A value of $P<.05$ was considered statistically different.

Results

Effects of Ang II on the Renal Microvasculature

In a series of preliminary studies, we characterized the temporal profile of renal microvascular constriction in response to the administration of Ang II. Ang II (0.3 nmol/L) decreased afferent arteriolar diameter by 37±5% (from 16.8±0.6 to 10.6±0.9 μm, n=5, $P<.005$). The Ang II–induced vasoconstriction persisted without change for >2.5 hours; 150 minutes after the addition of Ang II, afferent arteriolar diameter remained decreased by 35±7% (to 10.7±0.8 μm, $P>.50$ versus Ang II).

The renal microvascular responses to Ang II and isradipine are summarized in Fig 1. The addition of Ang II (0.3 nmol/L) elicited substantial afferent (Fig 1, left) arteriolar constriction (from 20.0±0.8 to 12.7±0.5 μm, n=12, $P<.001$). Subsequent administration of increasing doses of isradipine progressively reversed this constriction. Significant vasodilatation was observed at 0.1 nmol/L isradipine (to 14.4±0.5 μm, $P<.05$ versus Ang II). At 0.1 μmol/L, isradipine completely restored the afferent arteriolar diameter (to 20.0±0.8 μm, $P>.50$ versus basal value). At 1 μmol/L isradipine, afferent arteriolar diameter slightly exceeded its basal diameter (to 20.6±0.8 μm, $P<.05$ versus basal value).

Ang II (0.3 nmol/L) also induced considerable efferent (Fig 1, right) arteriolar constriction (from 16.4±0.8 to 12.0±0.4 μm, n=12, $P<.001$). The addition of isradipine (from 0.1 to 100 nmol/L) failed to alter the efferent arteriolar constriction induced by Ang II. At 1 μmol/L, isradipine slightly increased efferent arteriolar diameter (to 13.1±0.5 μm, $P<.005$ versus Ang II).

Effects of KCl on the Renal Microvasculature

Fig 2 depicts the effects of isradipine on KCl-induced afferent arteriolar constriction. The increase in potas-
constriction. Thus, the half-maximal inhibitory concentration (IC\textsubscript{50}) for isradipine was identical for the reversal of Ang II (1.2 ± 0.2 nmol/L)-induced and KCl (4.2 ± 0.9 nmol/L, P = NS versus Ang II)-induced afferent arteriolar constriction.

**Effects of Endothelin on the Renal Microvasculature**

Fig 4 summarizes the effects of isradipine on ET-induced renal microvascular constriction. ET (0.3 nmol/L) resulted in profound afferent (Fig 4, left) arteriolar constriction (from 18.7 ± 0.8 to 13.2 ± 0.6 μm, n = 19, P < .001). In contrast to Ang II, doses of isradipine equal to or exceeding 1 nmol/L were required to elicit significant vasodilation of the afferent arteriole (to 13.7 ± 0.6 μm, P < .05 versus ET). Furthermore, even at the highest dose used (1 μmol/L), isradipine only partially reversed afferent arteriolar constriction (to 17.5 ± 0.7 μm, P < .01 versus basal value).

The effects of isradipine on ET-induced efferent arteriolar constriction are depicted in Fig 4, right. ET (0.3 nmol/L) moderately constricted the efferent arteriole (from 15.9 ± 0.7 to 13.9 ± 0.8 μm, n = 8, P < .001). Isradipine (from 0.1 nmol/L to 1 μmol/L) failed to alter efferent arteriolar diameter.

**Studies With Staurosporine**

**Effects on endothelin-induced constriction.** To examine the contribution of protein kinase C to ET-induced constriction, the vessels were pretreated with staurosporine, a protein kinase C inhibitor. As shown in Fig 5, left, the addition of staurosporine in itself did not alter afferent arteriolar diameter. The subsequent administration of ET (0.3 nmol/L) substantially constricted afferent arterioles (from 19.2 ± 0.5 to 14.1 ± 0.4 μm, n = 14, P < .001). Treatment with increasing doses of isradipine reversed this vasoconstriction in a dose-dependent manner. In contrast to afferent arterioles untreated with staurosporine, doses as low as 0.1 nmol/L of isradipine were sufficient to induce significant vasodilation (to 15.2 ± 0.3 μm, P < .005). Under the condition of staurosporine pretreatment, isradipine (1
μmol/L) completely restored afferent arteriolar diameter to basal levels (to 19.2±0.5 μm, P>.50 versus basal value).

Fig 5, right, depicts the effects of isradipine and staurosporine on ET-induced efferent arteriolar constriction. Staurosporine did not induce significant changes in vessel diameter. ET (0.3 nmol/L) elicited moderate vasoconstriction (from 17.4±1.1 to 15.8±1.1 μm, n=10, P<.005). In contrast to the afferent arteriolar response, isradipine failed to attenuate ET-induced efferent arteriolar constriction.

Effects on KCl-induced constriction. Fig 6 summarizes the effects of isradipine and staurosporine on KCl-induced afferent arteriolar constriction. The administration of staurosporine did not alter afferent arteriolar diameter. Increasing the potassium concentration of the media reduced afferent arteriolar diameter by 35±5% (from 18.2±0.4 to 11.3±0.4 μm, n=9, P<.001). The subsequent addition of isradipine reversed KCl-induced afferent arteriolar constriction in a dose-dependent manner. In analogy with afferent arterioles not treated with staurosporine, isradipine in doses as low as 0.1 nmol/L induced significant vasodilation (to 12.9±0.3 μm, P<.05 versus KCl). At 0.1 and 1 μmol/L of isradipine, afferent arteriolar diameter returned to the basal level (18.1±0.5 and 18.4±0.5 μm, P>.50 versus basal value, respectively).

Fig 7 compares the effects of staurosporine on dose dependence to isradipine in reversing ET- and KCl-induced afferent arteriolar constriction. Data are expressed as percent inhibition of vasoconstriction. Without staurosporine pretreatment, ET-induced afferent arteriolar constriction was relatively resistant to isradipine as compared with that induced by KCl. Thus, the IC₅₀ of ET-induced afferent arteriolar constriction for isradipine (127±65 nmol/L) exceeded that for reversing KCl-induced vasoconstriction (4.2±0.9 nmol/L, P<.01). When the vessels were pretreated with staurosporine, however, isradipine readily reversed the afferent arteriolar constriction induced by ET in a manner identical to that induced by KCl. The IC₅₀ of ET-induced constriction of staurosporine-pretreated afferent arterioles for isradipine (4.3±1.7 nmol/L) was identical to that reversing KCl-induced constriction. In contrast to ET, KCl-induced afferent arteriolar constriction was similarly reversed by isradipine independent of staurosporine pretreatment. Thus, the IC₅₀ for isradipine for reversing KCl-induced constriction of afferent arterioles pretreated with staurosporine (3.6±1.5 nmol/L) was similar to that without staurosporine pretreatment.

![Figure 5](https://example.com/fig5.png)  
**Fig 5.** Line graphs show the effects of staurosporine and isradipine on endothelin-induced afferent (left) and efferent (right) arteriolar constriction. Pretreatment with staurosporine (50 nmol/L) did not alter either afferent or efferent arteriolar diameter. The subsequent administration of endothelin (0.3 nmol/L) constricted both afferent and efferent arterioles. The addition of increasing doses of isradipine reversed afferent arteriolar vasoconstriction in a dose-dependent manner. Indeed, at 1 μmol/L, isradipine had completely returned arteriolar diameter to control levels. However, isradipine failed to alter efferent arteriolar diameter. *P<.05 vs basal value. **P<.05 vs endothelin.

![Figure 6](https://example.com/fig6.png)  
**Fig 6.** Line graph shows the effects of staurosporine and isradipine on KCl (30 mEq/L)–induced afferent arteriolar constriction. Pretreatment with staurosporine (50 nmol/L) did not alter either afferent or efferent arteriolar diameter. The addition of KCl induced substantial afferent arteriolar constriction. The subsequent addition of increasing doses of isradipine reversed this afferent arteriolar vasoconstriction in a dose-dependent manner. At 0.1 μmol/L, isradipine completely reversed afferent arteriolar diameter. *P<.05 vs basal value. **P<.05 vs KCl.
FIG 7. Line graph compares dose dependence to isradipine in reversing endothelin-induced afferent arteriolar constriction. Data are shown for experiments with and without staurosporine. Without staurosporine pretreatment (A), endothelin-induced afferent arteriolar constriction was relatively resistant to isradipine as compared with that induced by KCl (○). When the kidneys were pretreated with staurosporine, however, isradipine readily reversed the afferent arteriolar constriction elicited by endothelin (▲) in a manner identical to that induced by KCl (○). However, staurosporine pretreatment did not alter the dose dependence to isradipine in reversing KCl-induced afferent arteriolar constriction (●).

Discussion

Signal transduction of vasoactive peptides, including Ang II and ET, has been investigated extensively, primarily using aortic vascular smooth muscle cells.22,23 These vasoconstrictor peptides have been demonstrated to bind to specific receptors on vascular smooth muscle cells and induce phosphatidylinositol breakdown.22,24 The increase in inositol triphosphate and/or calcium mobilization elicits the opening of chloride channels, depolarizes the membrane, and activates voltage-dependent calcium channels.23,25,26 The postreceptor mechanisms triggered by vasoconstrictor peptides are, however, not well defined in the renal microcirculation and may differ from those of aortic smooth muscle cells. Thus, the cellular mechanisms mediating renal microvascular constriction may differ with different constrictor peptides and at different sites of the renal microvasculature.

Ang II–induced afferent arteriolar constriction appears to depend on calcium entry through voltage-dependent calcium channels. In the present study, we have demonstrated that Ang II–induced afferent arteriolar constriction is completely reversed by the dihydropyridine isradipine, indicating the major contribution of voltage-dependent calcium channels in eliciting this vasoconstriction. Our observations are in accord with those of Carmines and Navar.10 Using the blood-perfused juxtaglomerular nephron, they reported that diltiazem and/or verapamil reversed the decrease in afferent arteriolar diameter induced by Ang II. We have extended these observations and have demonstrated that the dose dependence to isradipine in reversing Ang II–induced afferent arteriolar constriction is identical to that of KCl-induced constriction (Fig 3). It has been reported that high potassium medium exclusively activates voltage-dependent calcium channels.19,27 Collectively, our present studies support the previous observations by Carmines and Navar and further suggest that sustained Ang II–induced afferent arteriolar constriction is mediated primarily by calcium entry through voltage-dependent calcium channels.

ET-induced afferent arteriolar constriction also appears to depend, at least in part, on calcium entry through voltage-dependent calcium channels. We have demonstrated that isradipine reverses ET-induced afferent arteriolar constriction in a dose-dependent manner, indicating the involvement of voltage-dependent calcium channels in this vasoconstriction. Our results are consistent with the findings of Edwards et al,11 who demonstrated that constriction of isolated afferent arterioles induced by ET is mediated, at least in part, by voltage-dependent calcium channels. In contrast to Ang II, however, ET-induced afferent arteriolar constriction is only partially reversed by the highest concentration of isradipine used (1 μmol/L). Furthermore, our data indicate that ET-induced afferent arteriolar constriction, as compared with KCl-induced constriction, is relatively resistant to isradipine. The IC50 of ET-induced afferent arteriolar constriction for isradipine is greater than the IC50 of KCl-induced constriction (170 versus 1.2 nmol/L). Our results suggest that, in contrast to Ang II–induced afferent arteriolar constriction, ET-induced afferent arteriolar constriction may involve other mechanisms in addition to voltage-dependent calcium channels, thereby rendering it resistant to calcium antagonists.

Another explanation for these differences between Ang II and ET may be that the membrane potential induced by Ang II might be more positive than that induced by ET, thereby increasing the affinity of isradipine to calcium channels.28 Using vascular smooth muscle, Dacquet et al12 described that the affinity of isradipine to calcium channels in the resting state is five times less than in the inactivated state. Ruiz-Velasco et al29 also showed that the affinity of isradipine was increased fourfold after membrane depolarization. However, we have demonstrated that the IC50 for isradipine in inhibiting Ang II–induced constriction is 142 times lower than that of ET-induced constriction. Therefore, it appears less likely that this mechanism plays a major role in determining the differing IC50s between Ang II– and ET-induced afferent arteriolar constriction.

The activation of protein kinase C is known to induce slowly developing but sustained cellular responses.30 Using phorbol ester, Jiang and Morgan31 have suggested that the activation of protein kinase C increases the sensitivity of contractile elements to cytosolic calcium. In addition, it has been shown that ET activates protein kinase C in aortic strips.14 In concert, these observations raise the possibility that the resistance of ET-induced afferent arteriolar constriction to calcium antagonists may relate to protein kinase C activation.

To ascertain whether a protein kinase C–related mechanism(s) was involved in ET-induced renal microvascular constriction, the effects of isradipine on ET-induced renal microvascular constriction were assessed during the inhibition of protein kinase C by staurosporine, a protein kinase C inhibitor. In the present exper-
ments, we have demonstrated that, after pretreatment with staurosporine (50 nmol/L), isradipine reverses ET-induced afferent arteriolar constriction in a manner similar to that induced by KCl. Furthermore, the co-administration of staurosporine and isradipine (1 μmol/L) completely reversed the afferent arteriolar constriction elicited by ET. Consequently, the present results are consistent with the postulate that the activation of protein kinase C, in addition to voltage-dependent calcium channels, participates in mediating ET-induced afferent arteriolar constriction.

Staurosporine was selected as a pharmacological probe to inhibit protein kinase C because it is not light dependent and appears to be the most effective protein kinase C inhibitor available to date. We have shown that pretreatment with staurosporine did not induce any changes in either afferent or efferent arteriolar diameter. This suggests that the addition of staurosporine solution did not substantively alter membrane potential or permeability in the renal microvasculature. Additionally, Laher et al32 have reported that staurosporine (50 nmol/L) did not affect KCl-induced contraction. In accord with their results, we have demonstrated that KCl-induced afferent arteriolar constriction was not attenuated by staurosporine (50 nmol/L) pretreatment (33 ± 3% versus 35 ± 5%), suggesting that staurosporine does not appreciably inhibit myosin light chain kinase at this concentration. Furthermore, we observed that KCl-induced afferent arteriolar constriction was similarly reversed by isradipine independent of staurosporine pretreatment. These findings suggest that staurosporine did not interact nonspecifically with voltage-dependent calcium channels, supporting the specificity of this protein kinase C inhibitor.

The possible mechanisms whereby the inhibition of protein kinase C decreases the IC50 for isradipine in reversing ET-induced afferent arteriolar constriction merit comments. As mentioned above, one of the mechanisms could be that protein kinase C increases the sensitivity of contractile elements to calcium.30 Other mechanisms include the possibility that protein kinase C may alter the affinity of isradipine to voltage-dependent calcium channels by acting on the calcium channels themselves31 or possibly by an effect on the membrane potential.32 Since the substrates for protein kinase C in the afferent arteriole are not established, the precise post–protein kinase C mechanisms remain to be determined.

The addition of Ang II to the perfusate elicits efferent arteriolar constriction, which was relatively resistant to isradipine; only at the highest dose (1 μmol/L) of isradipine were we able to demonstrate a slight increase in efferent arteriolar diameter. Our present observation is consistent with previous observations that verapamil induces a modest efferent arteriolar vasodilation in the juxtamedullary nephron preparation.10

Although we cannot exclude the possibility that the present observations might relate to direct effects of calcium antagonists on the efferent arteriole, it is unlikely that the blockade of voltage-dependent calcium channels on efferent arterioles contributed appreciably to our results. We have previously demonstrated that KCl-induced depolarizing stimuli primarily constrict afferent arterioles.19 Consistent with our findings, Carmines et al35 demonstrated that high potassium stimuli did not increase cytosolic calcium concentration in efferent arterioles. Additionally, Steinhausen et al36 showed that the addition of Bay K 8644 failed to constrict postglomerular microvessels. These results suggest that voltage-dependent calcium channels are physiologically silent in the efferent arteriole. Furthermore, dihydropyridine calcium antagonists are more selective than diltiazem and verapamil in modulating voltage-dependent calcium channels.27,37

The mechanisms whereby the administration of isradipine increased efferent arteriolar diameter merit comment. In the present study, we observed that, in response to 1 μmol/L isradipine, afferent arteriolar diameter increased slightly, albeit significantly. It is tempting to speculate that perfusion pressure in the efferent arteriole would increase as a consequence of afferent arteriolar dilatation. This, in turn, might have resulted in a passive increase in the diameter of the efferent arteriole without a change in tension.

ET also induced a modest constriction of the efferent arteriole. The subsequent addition of increasing doses of isradipine failed to increase efferent arteriolar diameter, even though the efferent arteriolar constriction induced by ET was less prominent than the constriction induced by Ang II. These observations indicate that isradipine, at the concentrations used in the present study, does not modify efferent arteriolar constriction and suggest that ET-induced efferent arteriolar tension development is independent of voltage-dependent calcium channels.

In the present experiments, although pretreatment with staurosporine tended to decrease the magnitude of ET-induced efferent arteriolar constriction (12 ± 2% versus 9 ± 2%, P > .10), this effect did not attain statistical significance. Although staurosporine (50 nmol/L) has been used to block protein kinase C,14,32 we did not test the potency of this inhibitor using phorbol esters. Thus, the possibility remains that staurosporine might insignificantly inhibit protein kinase C in the efferent arteriole. Nevertheless, a consideration of the anatomic differences of the afferent and efferent arterioles renders the latter possibility unlikely. Vascular smooth muscle cells are in contact with each other more loosely in efferent arterioles than in afferent arterioles.38 Consequently, the penetration of the inhibitor into the efferent arteriole should exceed that attained in the afferent arteriole. We have already shown the effectiveness of identical doses of staurosporine in afferent arterioles, suggesting that such doses should be adequate to inhibit protein kinase C in the efferent arteriole.

Alternatively, the reasons for the above observations may relate to the inhibition of vasoconstriction by protein kinase C. The activation of protein kinase C decreases cytosolic calcium concentration by promoting the sequestration of calcium34; it also increases the sensitivity of contractile elements to calcium.31 Therefore, it is possible that the inhibition of protein kinase C might induce a further increase in cytosolic calcium in renal microvessels during the sustained phase of constriction by ET. Thus, an exaggerated response of cytosolic calcium in renal microvessels to ET during staurosporine treatment may account for the persistence of ET-induced constriction of the efferent arterioles pretreated with staurosporine.
Finally, a caveat is in order. The present experiments were performed using the isolated perfused hydropnephrotic kidney model because renal microvascular responsiveness is not assessable directly by videomicroscopy in the normal kidney. It has not been established, however, that the function of glomerular arterioles in hydropnephrotic kidneys is identical with that of normal kidneys. Nevertheless, we have demonstrated previously that the vasoconstrictor stimuli used in the present study produce quite similar renal hemodynamic responses in isolated perfused normal and hydropnephrotic kidneys.4,9,19,39 Thus, it is reasonable to assume that the responses observed in the hydropnephrotic kidney do not differ from those operative in the normal kidney.

In summary, the present data indicate that the sustained Ang II-induced afferent arteriolar constriction is primarily maintained by calcium entry through voltage-dependent calcium channels. Furthermore, our data suggest that ET-induced afferent arteriolar constriction is mediated by the activation of protein kinase C, in addition to voltage-dependent calcium channels. Finally, our results are consistent with the postulate that effenter arteriolar constriction induced by Ang II and ET is relatively independent of the activation of voltage-dependent calcium channels.

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