Calcium Mobilization Contributes to Pressure-Mediated Afferent Arteriolar Vasoconstriction

Edward W. Inscho, Anthony K. Cook, Vy Mui, John D. Imlay

Abstract—Preglomerular responses to vasoactive agonists utilize calcium released from intracellular stores and activation of calcium influx pathways to elicit vasoconstriction. The current study was performed to determine the role of calcium release from intracellular stores on the afferent arteriolar response to increases in perfusion pressure. Experiments were performed, in vitro, using the blood perfused, juxtamedullary nephron technique combined with videomicroscopy. The response of afferent arterioles to 30 mm Hg increases in perfusion pressure was determined before and after depletion of intracellular calcium pools with a 10-minute preincubation with 1 μmol/L thapsigargin or 100 μmol/L cyclopiamine acid. Afferent arteriolar diameter averaged 20.2 ± 1.0 μm (n = 19) at a control perfusion pressure of 100 mm Hg. Increasing perfusion pressure to 130 and 160 mm Hg reduced afferent caliber by 10.7 ± 1.0% (P < 0.05 versus con) and by 24.7 ± 1.6% (P < 0.05 versus diameter at 130 mm Hg), respectively. Thapsigargin significantly increased afferent diameter by 21.2% (n = 6) at 100 mm Hg and prevented pressure-induced autoregulatory responses. Afferent arteriolar diameter averaged 24.3 ± 1.7, 24.5 ± 1.8 and 24.3 ± 1.8 μm at perfusion pressures of 100, 130 and 160 mm Hg, respectively. Cyclopiamine acid treatment also inhibited autoregulatory behavior but did not alter resting vessel diameter. Afferent arteriolar diameter (n = 6) averaged 21.4 ± 1.9 μm at 100 mm Hg and 20.9 ± 2.1 and 20.5 ± 2.2 μm at 130 and 160 mm Hg, respectively. Additional studies were performed to assess the role of phosphohosphatase C activity in pressure-mediated autoregulatory behavior of afferent arterioles. Step increases in perfusion pressure decreased afferent diameter by 10.7 ± 3.8 and 21.7 ± 4.1%, respectively. Administration of the phosphohosphatase C inhibitor, U 73122, (5 μmol/L) did not significantly alter baseline diameter but did attenuate the pressure-mediated vasoconstrictor response. Increasing perfusion pressure to 130 and 160 mm Hg reduced afferent arteriolar diameter by only 6.5 ± 1.5 and 10.6 ± 2.0%, respectively. These data demonstrate that interruption of calcium mobilization with thapsigargin, cyclopiamine acid, or phosphohosphatase C inhibition markedly attenuates pressure-mediated afferent arteriolar vasoconstriction and suggest that autoregulatory adjustments in afferent arteriolar diameter involve calcium release from inositoltrisphosphate(IP3)-sensitive intracellular stores. (Hypertension. 1998;31[part 2]:421-428.)

Key Words: calcium mobilization ■ thapsigargin ■ cyclopiamine acid ■ autoregulation ■ phosphohosphatase C ■ U 73122 ■ microcirculation ■ renal

One of the main functions of the preglomerular microvasculature is to regulate glomerular capillary pressure by adjusting afferent arteriolar resistance in response to changes in perfusion pressure. This autoregulatory behavior represents the combined influences of both myogenic and tubuloglomerular feedback (TGF) generated alterations in microvascular wall tension regulating preglomerular resistance. While some aspects of autoregulation have been delineated, the intracellular signal transduction events responsible for pressure-dependent adjustments in preglomerular resistance remain poorly understood. Previous studies have shown that vascular resistance changes evoked in response to alterations in renal perfusion pressure primarily reflect adjustments in afferent arteriolar diameter. Elevation of cytosolic calcium concentration represents an important component in the afferent arteriolar response to perfusion pressure changes. Pressure-mediated vasoconstriction of afferent arterioles relies heavily on the influx of extracellular calcium through voltage-gated calcium channels, although the nature of the depolarizing stimulus remains to be identified. Also unclear is the role of calcium release from intracellular stores in the vascular response to mechanical stimulation.

Pressure-mediated elevations of cytosolic calcium concentration in vascular smooth muscle can occur through activation of calcium influx pathways involving stretch-activated, nonselective cation channels, membrane depolarization, and activation of potential dependent ion channels, and/or through mobilization of calcium release from intracellular pools. Studies in nonrenal vascular preparations have shown that mechanical stimuli evoke rapid increases in cytosolic calcium concentration. Although still controversial, some studies suggest that part of the increase in intracellular calcium concentration derives from calcium released from intracellular pools. Other studies have shown that increases in transmembrane pressure stimulate phosphohosphatase C and phosphohosphatase D activity leading to generation of IP3 and diacylgly-

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cerol, whereas others implicate a role for pressure-mediated activation of protein kinase C \(^{23,29,31,33}\). Finally, it has recently been reported that rapid stretching of individual vascular smooth muscle cells stimulates a rapid increase in cytosolic calcium concentration partly through release of calcium from intracellular stores.\(^{37}\) Thus, pressure-mediated calcium release from intracellular stores may represent an important step in the sequence of events coupling changes in transmural pressure and the subsequent myogenic vasoconstriction shown to occur in vascular smooth muscle from most vascular beds.

With the recent development of agents which selectively perturb the filling state of intracellular calcium pools, it is now possible to manipulate these pools and determine the influence of calcium store integrity on microvascular responsiveness to acute changes in perfusion pressure.\(^{35-39}\) In addition, new enzyme inhibitors selective for steps of the intracellular calcium response pathway,\(^\text{23,60}\) allow interruption of specific components of the phospholipase C/calcium release cascade in order to determine their role in mediating pressure-dependent renal microvascular responses.

The current studies were performed to determine the effect of calcium store depletion on the afferent arteriolar response to acute changes in perfusion pressure. Direct assessment of afferent arteriolar responsiveness to step increases in perfusion pressure was performed before and during depletion of intracellular calcium pools with the Ca\(^{2+}\)-ATPase inhibitors, thapsigargin and cyclopiazonic acid. Thapsigargin and cyclopiazonic acid have been shown to deplete IP\(_3\)-sensitive intracellular calcium stores, thereby interrupting IP\(_3\)-dependent, agonist-mediated responses.\(^{35-39}\) In addition, studies were performed to determine the effect of phospholipase C inhibition, with U-73122, on the afferent arteriolar response to increases in perfusion pressure.

### Methods

#### Materials

Enalaprilat was a gift from Merck Sharp and Dohme NE (Levophed) and was obtained from Winthrop Pharmaceuticals. U-73122 was purchased from BIOMOL Research Laboratories Inc. Thapsigargin was purchased from Research Biochemicals Inc. Cyclopiazonic acid and all other reagents were purchased from Sigma Chemical Company.

#### Kidney Preparation

Studies were performed as approved by the Tulane University Advisory Committee for Animal Resources. Experiments were conducted in vitro using the blood perfused juxtamedullary nephron technique as previously described.\(^ {41,42}\) For each experiment, two male Sprague-Dawley rats (350 to 400 g) were anesthetized with sodium pentobarbital (40 mg/kg, IP) and pretreated (30 minutes) with the converting enzyme inhibitor, enalaprilat (2 mg, IV). Perfusate blood was collected from the kidney and blood donors and was prepared as previously described.\(^ {42}\) The blood donor rat was nephrectomized and, 30 minutes later, exsanguinated into a syringe containing heparin (500 units). The plasma and erythrocyte fractions were separated from the centrifuged blood and the leukocyte fraction was discarded. The plasma was filtered (0.2 \(\mu\)m) before being mixed with erythrocytes to yield a hematocrit of approximately 0.33. The reconstituted blood was filtered through a 5 \(\mu\)m nylon mesh.

The right renal artery of the kidney donor was cannulated, and the kidney was anesthetically perfused with a Tyrode's buffer solution containing 52.0 g/L bovine serum albumin and a complement of L-amino acids.\(^ {42}\) The perfused kidney was removed and sectioned longitudinally to leave the papilla intact on the dorsal two-thirds portion of the kidney. The papilla was reflected back, and the pelvic mucosa was removed to expose the renal arterial branches, renal tubules, glomeruli, and related microvasculature of juxtamedullary nephrons. Ligation of the terminal ends of the large arteries restored perfusion pressure to the perfused cortical and papillary tissue. When the dissection was completed, the cell-free perfusate was replaced with the reconstituted, oxygenated blood. Perfusion pressure was monitored at the cannula tip and was set at 100 mm Hg. The inner cortical surface of the kidney was continuously superfused with warmed (37°C) Tyrode's buffer containing 10.0 g/L bovine serum albumin, and the kidney was allowed to equilibrate for at least 15 minutes.

The perfusion chamber containing the prepared kidney was affixed to the stage of a Nikon Xenophot microscope (Nikon Corp.) equipped with long working distance objectives. Video images, generated with a high resolution Newvicon camera (NC-67m, Dage-MTI) were electronically enhanced (image processor MFJ-1425, MFJ Enterprises Inc.) and displayed on a video monitor while being simultaneously recorded on videotape for analysis. Afferent arteriolar inside diameters were measured at a single site using an image-sampling monitor (Model 901, Instrumentation for Physiology and Medicine) calibrated with a stage micrometer. The steady-state diameter of each arteriole was calculated for each experimental period by averaging the measurements taken during the last 1 or 2 minutes of that period. Afferent arterioles were selected for study based on the clarity of the vascular walls and the adequacy of blood flow through the vessel lumen.

### Autoregulation Protocols

#### Effect of Thapsigargin or Cyclopiazonic Acid on the Afferent Arteriolar Response to Increasing Perfusion Pressure

The effect of increasing perfusion pressure on afferent arteriolar diameter was determined before and after calcium pool depletion with either thapsigargin or cyclopiazonic acid. Thapsigargin is a sequent lactone compound, which has been shown to specifically inhibit Ca\(^{2+}\)-ATPase from the endo- and sarcoplasmic reticulum.\(^ {39-40}\) Cyclopiazonic acid is a imidazole tetramic acid, produced by Aspergillus or Penicillium, which has also been shown to be a selective inhibitor of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase.\(^ {38,43}\) The thapsigargin (1 \(\mu\)M) and cyclopiazonic acid (100 \(\mu\)M) concentrations were chosen based on previous studies demonstrating that these concentrations were maximally effective in transiently increasing cytosolic calcium coincident with intracellular store depletion.\(^ {37}\) In addition, previous studies have shown that 1 \(\mu\)M thapsigargin markedly attenuates afferent and efferent arteriolar responsiveness to angiotensin II\(^ {42}\) and norepinephrine.\(^ {42}\)

The following autoregulatory experiments were performed. Afferent arteriolar diameter was determined during the 5-minute control period with perfusion pressure set at 100 mm Hg. Subsequently, perfusion pressure was increased in 30 mm Hg increments, to 130 mm Hg and 160 mm Hg, in consecutive 3-minute periods. Afferent arteriolar diameter was monitored constantly during each pressure step, and the steady-state diameter was determined by computing the mean diameter during the final minute. Finally, perfusion pressure was returned to 100 mm Hg and a 5-minute recovery period ensued. The role of intracellular calcium stores in the afferent arteriolar response to increasing perfusion pressure was assessed by treating each arteriole with thapsigargin (1 \(\mu\)M) or cyclopiazonic acid (100 \(\mu\)M) for 10 minutes before increasing perfusion pressure again to 130 mm Hg and 160 mm Hg. Perfusion pressure was then returned to 100 mm Hg for a 5-minute recovery period.

#### Selected Abbreviations and Acronyms

- TGF = tubuloglomerular feedback
- IP\(_3\) = inositol 1,4,5-trisphosphate
- Ca\(^{2+}\) = calcium ion
- Ca\(^{2+}\)-ATPase = calcium ion ATPase
- PKC = protein kinase C
- PKG = protein kinase G
- IP\(_3\) = inositol 1,4,5-trisphosphate
- L-AMPA = L-amino acid
- Ca\(^{2+}\)-ATPase = calcium ion ATPase
- NMDA = N-methyl-D-aspartate
Figure 1. Effect of thapsigargin on the afferent arteriolar response to increasing perfusion pressure. A, Changes in afferent diameter are expressed in microns under control conditions (open circles) and during thapsigargin treatment (THAPS, closed circles). The time scales for the control and thapsigargin responses are overlapped for comparison. B, Changes in afferent diameter are expressed as a percentage of the control diameter. Afferent arteriolar responses to perfusion pressures of 100, 130, and 160 mm Hg are shown during the control conditions (open circles) and during thapsigargin treatment (THAPS, closed circles). n = 6 afferent arterioles from 6 kidneys * = P < 0.05 versus control diameter, † = P < 0.05 versus thapsigargin treatment.

Figure 2. Effect of cyclopiazonic acid on the afferent arteriolar response to increasing perfusion pressure. A, Changes in afferent diameter are expressed in microns under control conditions (open circles) and during cyclopiazonic acid treatment (CPA, closed circles). The time scales for the control and cyclopiazonic acid responses are overlapped for comparison. B, Changes in afferent diameter are expressed as a percentage of the control diameter. Afferent arteriolar responses to perfusion pressures of 100, 130, and 160 mm Hg are shown during the control conditions (open circles) and during cyclopiazonic acid treatment (CPA, closed circles). n = 6 afferent arterioles from 6 kidneys * = P < 0.05 versus control diameter, † = P < 0.05 versus cyclopiazonic acid treatment.

Effect of the Phospholipase C inhibitor, U73122 on the Afferent Arteriolar Response to Increasing Perfusion Pressure

A second series of experiments was performed to determine the role of phospholipase C in the afferent arteriolar response to increasing perfusion pressure. For these experiments, afferent arteriolar diameter was determined during the 5-minute control period and during the subsequent 3-minute periods at 130 mm Hg and 160 mm Hg, respectively. Following the 5-minute recovery period, the phospholipase C inhibitor, U73122 (5 μmol/L), was administered via the superfusion solution, and the arterioles were allowed to equilibrate for a minimum of 20 minutes. Following the equilibration period, afferent arteriolar diameter was again measured at perfusion pressures of 100 mm Hg, 130 mm Hg, and 160 mm Hg before the final recovery period. As previously stated, vessel diameter was monitored continuously during the entire protocol.

Statistical Analysis

Statistical comparisons within each series were made using a one-way analysis of variance for repeated measures combined with the Newman-Keuls multiple range test. A probability value of P < 0.05 was considered significant. All data are reported as the mean ± SE.

Results

A total of 19 juxtamedullary afferent arterioles were used for these studies. These arterioles averaged 454 ± 30 μm in length and 20.2 ± 10 μm in diameter. Vascular diameter measurements were made an average of 220 ± 15 μm from the glomerulus representing the midpoint of the vessel length. There was no significant difference between the resting afferent arteriolar diameter, length, or measurement distance from the glomerulus between any of the treatment groups.

Thapsigargin Studies

The effect of thapsigargin on the afferent arteriolar response to increasing perfusion pressure is shown in Fig 1. Six afferent arterioles averaged 20.0 ± 1.2 μm during the control period and this diameter decreased reversibly in response to consecutive 30 mm Hg increases in perfusion pressure (Fig 1A). Afferent caliber decreased significantly to 18.0 ± 1.2 and 15.6 ± 1.2 μm at perfusion pressures of 130 mm Hg and 160 mm Hg, respectively. This represents a decline in afferent diameter to 90 ± 1% and 78 ± 2% of control, respectively (Fig 1B). Returning perfusion pressure to 100 mm Hg resulted in a complete reversal of the pressure-mediated vasoconstriction. Thapsigargin treatment resulted in a significant relaxation of these afferent arterioles with vessel diameter increasing by 21 ± 2% and abolished the pressure-dependent vasoconstriction observed under control conditions. Afferent arteriolar diameter averaged 24.3 ± 1.7, 24.5 ± 1.8, 24.3 ± 1.8 μm at perfusion pressures of 100 mm Hg, 130 mm Hg, and 160 mm Hg, respectively, corresponding to 101 ± 1% and 100 ± 1% of the diameter with thapsigargin alone (Fig 1B).

Cyclopiazonic Acid Studies

The effect of cyclopiazonic acid on the afferent arteriolar response to perfusion pressure was also examined and the...
The results of those studies are illustrated in Fig 2. The diameter of six afferent arterioles averaged 21.4 ± 2.1 μm during the control period, and this diameter decreased in a pressure-dependent manner in response to consecutive step increases in perfusion pressure to 130 mm Hg and 160 mm Hg. Afferent caliber decreased significantly to 19.1 ± 2.1 and 15.6 ± 1.6 μm, respectively. Cyclosporine acid treatment transiently vasoconstricted the afferent arterioles. Vessel diameter decreased to a minimum diameter of 19.7 ± 2.1 μm within approximately 1 minute of exposure before gradually recovering to a diameter similar to control (21.4 ± 2.0 μm). In the continued presence of cyclosporine acid, the afferent arteriolar diameter response to increasing perfusion pressure was reassessed. Whereas increasing perfusion pressure to 130 mm Hg and 160 mm Hg reduced afferent diameter to 89 ± 2% and 73 ± 2% of control under the control conditions (Fig 2B), in the presence of cyclosporine acid, afferent caliber remained essentially unchanged. Afferent diameter averaged 21.4 ± 2.1 and 20.5 ± 2.2 μm at perfusion pressures of 130 mm Hg and 160 mm Hg, respectively. These values represent diameters of 98 ± 1% and 95 ± 2% of the diameter with cyclosporine acid alone (Fig 2B).

**U-73122 Studies**

Studies were also performed to assess the role of phospholipase C activity in pressure-mediated afferent arteriolar autoregulatory behavior. These studies were performed by assessing pressure-dependent changes in afferent arteriolar diameter before and during administration of the phospholipase C inhibitor, U-73122. The results of those experiments are illustrated in Fig 3. The control diameter of seven afferent arterioles averaged 20.5 ± 9 μm (Fig 3A). Increasing perfusion pressure in two 30 mm Hg increments decreased vessel diameter to 89 ± 4% and 78 ± 4% of the control diameter (Fig 3A and 3B). Arteriolar diameter returned to the control diameter (20.5 ± 0.9) when perfusion pressure was reset to 100 mm Hg. Administration of 5 μmoles/L U-73122 did not significantly alter baseline afferent arteriolar diameter. With perfusion pressure set at 100 mm Hg, afferent diameter averaged 19.9 ± 7 μm, which is 99 ± 3% of the starting diameter. In the presence of U-73122, the pressure-mediated afferent arteriolar vasoconstrictor response was reassessed (Fig 3A and 3B). Increasing perfusion pressure to 130 mm Hg and 160 mm Hg reduced afferent diameter significantly to 94 ± 2% and 90 ± 2% of the baseline diameter at 100 mm Hg; however, as shown in Fig 3B, the magnitude of the response at 160 mm Hg was significantly attenuated, compared to the control response.

**Discussion**

In the present study, we directly examined the effect of intracellular calcium store depletion and phospholipase C inhibition on the afferent arteriolar response to increases in perfusion pressure. The results of these studies demonstrate that pressure-mediated autoregulatory adjustments in juxtaglomerular afferent arteriolar diameter involve release of Ca²⁺ from purported IP₃-sensitive, intracellular calcium stores. Consistent with this observation is the finding that pressure-mediated adjustments in afferent arteriolar diameter are adversely affected by inhibition of phospholipase C activity. Therefore, phospholipase C activation with the subsequent generation of IP₃ appears to be an important component in the sequence of events culminating in autoregulatory adjustments in renal vascular resistance.

The kidney is capable of precisely regulating renal blood flow and glomerular filtration rate over a wide range of renal perfusion pressures. Exactly how renal autoregulation is accomplished has been the focus of intensive investigation, yet many aspects of the phenomenon remain to be explained. Nevertheless, several components of the autoregulatory response have been resolved. There is a general consensus that whole kidney autoregulatory behavior is a manifestation of the intrinsic myogenic behavior of the microvasculature supplemented by the vasoregulatory influences of the TGF mechanism of the juxtaglomerular apparatus. Direct evidence for the contribution of myogenic influences in the renal microvascular autoregulatory response comes from studies performed in the hydronephrotic kidney preparation, which maintains an intact renal microvascular network in the absence of renal tubules or juxtaglomerular apparatus. Microdissected afferent arterioles, or preparations in which TGF-dependent responses have been inhibited, evidence for the contribution of the TGF component comes from micropuncture studies where changes in distal tubular perfusion result in changes in pregglomerular resistance without any change in renal perfusion pressure and from isolated kidney preparations where loop perfusion and macula densa function can be manipulated by Myogenic vasoconstriction purportedly involves activation of stretch-activated, nonselective cation channels; fol-
lowed by depolarization and activation of voltage-gated calcium channels. Both TGF-mediated and myogenic adjustments in afferent arteriolar resistance can be blocked by L-type calcium channel antagonists thus attesting the crucial role voltage-dependent calcium influx plays in the autoregulatory response.

The role of calcium release from intracellular stores is more controversial. Earlier studies suggested that calcium mobilization from intracellular stores is not a major contributor to whole kidney autoregulatory responses in the dog. However, studies performed in other tissues exhibiting autoregulatory behavior suggest that calcium mobilization from intracellular stores may represent an important component in the overall response. D'Angelo and Memminger presented a flow scheme describing the critical elements involved in myogenic responses of vascular smooth muscle. Accordingly, vascular smooth muscle responses to mechanical stretch include G-protein-dependent activation of phospholipase C with the subsequent generation of IP_3, leading to calcium mobilization from intracellular stores. More recently, Davis and coworkers reported that mechanical stretch of isolated coronary artery smooth muscle cells leads to the release of stored calcium into the cytosol. While this observation supports a role for calcium mobilization as part of the myogenic response to stretch, similar observations have been made from small caliber arterioles (<20 μm) in renal microvascular smooth muscle cells.

Thapsigargin and cyclopiazonic acid inhibit the activity of the sarcoplasmic reticulum Ca^{2+} ATPase. Calcium ATPase inhibition results in depletion of intracellular calcium stores as they are emptied via endogenous leakage pathways without being replenished through the activity of the sarcoplasmic reticulum Ca^{2+} ATPase. This eliminates the availability of a readily mobilizable calcium reservoir to be accessed by store-dependent vasoactive stimuli even in the presence of normal extracellular calcium concentrations. We have previously shown that thapsigargin treatment markedly attenuated the vasoconstriction of juxtaglomerular afferent arterioles induced by angiotensin II or norepinephrine. In the current report, both thapsigargin and cyclopiazonic acid markedly attenuated the afferent arteriolar vasoconstriction-induced by acute increases in perfusion pressure. This suggests that pressure-mediated calcium release from intracellular stores may be an essential step in the sequence of events coupling the increase in transmural pressure to the subsequent autoregulatory vasoconstriction.

Thapsigargin and cyclopiazonic acid have been used extensively as Ca^{2+} ATPase inhibitors for the depletion of intracellular calcium stores in a broad range of tissues and cell types. Nevertheless, there are reports that these agents may have other effects that may complicate interpretation of data obtained using them. Studies have suggested that thapsigargin and cyclopiazonic acid may also stimulate Ca^{2+} influx and directly cause vasoconstriction by elevating cytosolic calcium and causing membrane depolarization. In vascular smooth muscle, cellular influx is reported to occur through activation of L-type calcium channels as well as stimulating another dihydropyridine-insensitive pathway. Other studies have suggested that use of these agents results in drug-induced relaxation through inhibition of voltage-activated calcium channels or through activation of Ca^{2+}-activated K^+ channels following an elevation of cytosolic calcium concentration. Nevertheless, the possibility that such nonspecific effects may have invalidated or confounded our findings in the current report is unlikely. Both thapsigargin and cyclopiazonic acid completely eliminated pressure-mediated vasoconstriction of afferent arterioles. For two structurally dissimilar compounds to alter pressure-mediated autoregulatory behavior through an identical nonspecific effect is doubly especially when qualitatively similar results are obtained by inhibiting phospholipase C activity.

The possibility that three interventions designed to reduce calcium mobilization from intracellular stores would all nonspecifically compromise autoregulatory behavior is improbable. Furthermore, the actions of these Ca^{2+} ATPase inhibitors are not totally consistent with the reported nonspecific actions for these agents. For example, if thapsigargin was interfering with calcium influx through voltage-gated calcium channels, it would result in attenuation or blockade of pressure-mediated autoregulatory adjustments in afferent arteriolar diameter. However, we have previously demonstrated that 1 μmol/L thapsigargin does not inhibit the afferent arteriolar vasoconstriction induced by membrane depolarization with 55 mmole/L KCl. Similarly, Bay K 8644, has been shown to vasodilate afferent arterioles in the presence of cyclopiazonic acid. Retention of KCl-mediated and Bay K 8644-mediated afferent arteriolar vasoconstriction in the presence of thapsigargin or cyclopiazonic acid, respectively, argues against the nonspecific interaction of these agents with L-type calcium channels. In the present study, thapsigargin was found to increase afferent arteriolar diameter. This vasodilation is consistent with activation of Ca^{2+} influx. Cyclopiazonic acid had an identical effect on the autoregulatory response compared to thapsigargin but did not alter afferent arteriolar diameter. Therefore, in the presence of these Ca^{2+} ATPase inhibitors, the ability of calcium channels to alter afferent arteriolar caliber is retained.

Finally, Ca^{2+}-dependent activation of K^+ channels with the subsequent hyperpolarization of afferent arteriolar smooth muscle is also unlikely, since this would lead to vasorelaxation. In the current report, only thapsigargin had a significant effect on resting afferent arteriolar diameter. Thapsigargin treatment increased vascular diameter, whereas afferent diameter was unchanged in the presence of cyclopiazonic acid and tended to decrease slightly during inhibition of phospholipase C. It is doubtful that all three agents could be inhibiting autoregulatory behavior by activation of Ca^{2+}-dependent K^+ channels while each agent exerts a different effect on resting afferent arteriolar diameter. Accordingly, rather than nonspecific effects being responsible for the results obtained, these data strongly suggest that direct inhibition of the sarcoplasmic reticulum Ca^{2+} ATPase by thapsigargin and cyclopiazonic acid eliminated a crucial intracellular calcium source required to sustain the sequence of signal transduction events leading to an appropriate pressure-dependent vasoconstrictor response. Therefore, the data presented here support the hypothesis that thapsigargin and cyclopiazonic acid treatment depletes important afferent arteriolar calcium stores and interrupts a pivotal
component of the signal transduction cascade induced by acute changes in perfusion pressure

If changes in perfusion pressure and thus changes in microvascular transmural pressure stimulate autoregulatory responses involving calcium mobilization from IP₃-sensitive storage pools, then this response should involve activation of phospholipase C. Therefore, inhibition of phospholipase C activity should attenuate, or abolish, the microvascular response to increases in perfusion pressure. The results in the current report are consistent with this hypothesis. Phospholipase C inhibition, with U-73122, significantly attenuated the afferent arteriolar response to increased perfusion pressure. The magnitude of the attenuation appears to be smaller than the response obtained with thapsigargin or cyclopiazonic acid, and it may reflect incomplete blockade of phospholipase C activity or it may indicate that other second messenger sources or pathways also participate in the mobilization of intracellular Ca²⁺, such as cyclic ADP-ribose or tyrosine kinase. Nevertheless, attenuation of IP₃ formation by the phospholipase C inhibitor, U-73122, resulted in a clear attenuation of the pressure-dependent autoregulatory response of juxtamedullary afferent arterioles.

As previously stated, whole kidney autoregulation occurs through the combined influences of both TGF and myogenic regulation of microvascular function. The results of the current study do not allow us to identify which of these two regulatory systems have been manipulated by these pharmacological agents. Evidence suggests that the TGF mechanism exerts the greatest influence on preglomerular resistance along portions of the afferent arteriole nearest to the glomerulus, whereas myogenic regulation is most prominent at sites more distant from the glomerulus. In this report, afferent arteriolar diameter measurements were obtained near the midpoint (49±1%) of the vessel length. Evidence suggests that this region is subject to regulation by both myogenic and TGF signals. Nevertheless, it is interesting to note that despite the absence of pressure-mediated vasoconstriction during thapsigargin, cyclopiazonic acid, or U-73122 treatment, these afferent arterioles did maintain a relatively stable luminal diameter. Therefore, each increment in perfusion pressure still elicited an increase in vascular wall tension sufficient to prevent the arteriolar caliber from increasing passively with each step pressure, yet insufficient to reduce luminal diameter below control. Retention of some tension generating capacity in response to increases in perfusion pressure suggests that inhibition of calcium release from intracellular stores may primarily impact on just one of the two branches of overall renal autoregulatory behavior. This is supported by the studies focusing on the role of voltage-dependent calcium channels in autoregulation. Voltage-gated calcium channels play a major role in both TGF and myogenic autoregulatory adjustments in preglomerular resistance. In the presence of L-type Ca²⁺ channel antagonists, step increases in perfusion pressure coincide with passive, pressure-dependent increases in arteriolar diameter. The ability of calcium channel blockers to result in a passive afferent arteriolar diameter relationship with alterations in perfusion pressure is largely a reflection of the important role L-type calcium channels play in the regulation of renal microvascular function and responsiveness to changes in perfusion pressure. The results in the current report are consistent with this hypothesis. Phospholipase C inhibition, with U-73122, significantly attenuates pressure-mediated juxtamedullary afferent arteriolar responses by direct actions on the macula densa. They do not, however, allow differentiation of the extent of involvement of calcium mobilization in myogenic versus TGF-dependent autoregulatory responses. Further investigation of this possibility requires that pressure-mediated afferent arteriolar responses be assessed under feedback-dependent and -independent conditions.

In summary, depletion of intracellular calcium stores with thapsigargin or cyclopiazonic acid markedly attenuates juxtamedullary afferent arteriolar vasoconstrictor responses induced by acute increases in perfusion pressure. In addition, inhibition of phospholipase C activity significantly attenuates pressure-mediated arteriolar responses by direct actions on the macula densa. They do not, however, allow differentiation of the extent of involvement of calcium mobilization in myogenic versus TGF-dependent autoregulatory responses. Further investigation of this possibility requires that pressure-mediated afferent arteriolar responses be assessed under feedback-dependent and -independent conditions.
ated afferent arteriolar vasoconstrictor responses. These observations are in good agreement with the findings of other investigators using arteries isolated from other vascular beds that exhibit myogenic or autoregulatory behavior well as with in vivo studies involving whole kidney hemodynamics. The results of this study support the hypothesis that calcium release from an IP3-sensitive intracellular calcium pool represents a critical link in the signal transduction pathway responsible for autoregulatory adjustments in afferent arteriolar diameter.

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Autoregulatory Responses Require Intracellular Calcium Stores


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