Biophysical Signals Underlying Myogenic Responses in Rat Interlobular Artery

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Abstract—To assess cellular mechanisms mediating myogenic responses of interlobular artery (ILA), experiments were performed with the use of isolated perfused hydronephrotic kidneys. ILAs were divided into 3 groups according to their basal diameters: proximal (>60 μm), intermediate (40 to 60 μm), and distal (<40 μm) ILAs. Myogenic responses were obtained by stepwise increase in perfusion pressure. Greater myogenic responsiveness was observed in ILAs with smaller diameters. Diltiazem (10 μmol/L) inhibited myogenic responses of all segments of ILAs. Furthermore, gadolinium (10 μmol/L), a mechanosensitive cation channel blocker, abolished myogenic responses of distal but not proximal ILA. In contrast, 2-nitro-4-carboxyphenyl-N,N-diphenyl-carbamate (200 μmol/L), an inhibitor of phospholipase C, prevented myogenic responses of proximal but not distal ILA. Finally, basal proximal ILA diameters were increased by treatment with 50 nmol/L of staurosporine (P<0.05), and subsequent addition of thapsigargin (1 μmol/L) blocked myogenic contraction of proximal ILAs. Myogenic responses of intermediate ILAs exhibited characteristics between those of distal and proximal ILAs. Our data indicate that underlying mechanisms for myogenic responses differ in distinct segments of ILAs. The present results suggest that mechanosensitive cation channels are involved in myogenic constriction of distal ILAs. Finally, our findings provide evidence that the stimulation of phospholipase C mediates myogenic contraction of proximal ILAs. (Hypertension. 1998;32:1060-1065.)

Key Words: phospholipases ■ calcium ■ protein kinase C ■ membrane potential ■ calcium channels

An elevation of transmural pressure elicits the increase in tension of vessel wall. Numerous studies have indicated that this myogenic response plays an important role in regulation of capillary blood flow and pressure. There is still inconsistency regarding the mechanisms mediating myogenic responses. On the one hand, previous studies demonstrated that increasing pressure stimulates phospholipase C, thereby mobilizing intracellular calcium and mediating arterial myogenic response. On the other hand, arteriolar myogenic constriction depends on extracellular calcium.

Kidneys exhibit remarkable capacity to maintain constant blood flow despite marked variations in blood pressure. Recent results indicate that renal autoregulatory capacity was diminished in various pathophysiological conditions, including hypertension, diabetes mellitus, and progressive renal diseases. Furthermore, deranged renal autoregulation allows direct transmission of systemic blood pressure to glomeruli, thereby further worsening renal injury. Thus, the elucidation of mechanisms mediating autoregulation would be of great interest and could provide a theoretical framework for developing therapeutic strategies against chronic renal diseases. Carmines et al demonstrated that increasing pressure constricted all segments of preglomerular vessels, including afferent arteriole, interlobular artery (ILA), and arcuate artery. In addition to tubuloglomerular feedback (TGF), intact myogenic constriction is required for efficient autoregulation of glomerular blood flow. The afferent arteriole, which is the target of TGF, plays the most important role in autoregulatory adjustments of renal vascular resistance, but myogenic responses of ILAs also substantively participate in total renal autoregulation. While recent studies have validated that endothelium is not required for pressure-induced renal vasoconstriction, there is a paucity of data regarding mechanisms mediating myogenic response of ILAs.

In the present study experiments were performed with the use of isolated perfused hydronephrotic kidney model devoid of complex interactions between TGF and myogenic response. The effects of gadolinium, a potent mechanosensitive cation channel blocker, and 2-nitro-4-carboxyphenyl-N,N-diphenyl-carbamate (NCDC), an inhibitor of phospholipase C, were assessed. The present results indicate that myogenic responses are mediated by differing mechanisms in distinct segments of ILAs. Our data indicate that NCDC blocked myogenic response of proximal ILAs, providing evidence that myogenic response in proximal...
ILAs is mediated by the stimulation of phospholipase C. Our findings that myogenic constriction of distal ILAs was inhibited by either gadolinium or diltiazem suggest that pressure gates mechanosensitive cation channels, thereby activating voltage-dependent calcium channels in distal ILAs.

Methods

Gadolinium was purchased from Aldrich. Diltiazem and NCDC were obtained from Sigma. Thapsigargin and staurosporine were obtained from Research Biochemical.

Experiments were performed as described previously. In brief, hydropnephrosis was induced in 19 adult male Sprague-Dawley rats (Charles River Japan, Atsugi, Kanagawa) by ligating the right ureter under ether anesthesia. Eight to 10 weeks after the surgery, when renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of myogenic responses along the entire length of intact ILAs (Figure 1), the right kidney was harvested for perfusion study. ILA diameters were estimated with an automated program custom designed to determine the mean distance between parallel vessel walls. Three separate segments, ≈10 to 50 μm in length, were chosen from an ILA. As described previously, the segments of ILAs were divided into 3 groups according to their basal diameters at 80 mm Hg: proximal (>60 μm), intermediate (40 to 60 μm), and distal (<40 μm) ILAs.

In the first series of experiments (5 kidneys), initially basal myogenic responses were observed. Subsequently, diltiazem was added to achieve a final concentration of 10 μmol/L. Pressure challenge was again performed. Finally, potassium concentration in the perfusate was isosmotically increased to 30 mmol/L.5

In the second series of studies, the kidneys (n = 5) were exposed to 10 μmol/L of gadolinium after basal myogenic responses were assessed.3 In the presence of gadolinium, pressure challenge was performed, after which NCDC (200 μmol/L) was added.16 Thirty minutes later, myogenic responses were assessed. Finally, pressure challenge was performed. This concentration of NCDC was selected because it abolished Al/NaF-induced renal vasoconstriction under our experimental conditions.16

In the fourth studies (5 kidneys), basal myogenic responses were examined, then 50 mmol/L of staurosporine was added.19 Thirty minutes later, pressure challenge was performed. Subsequently, thapsigargin (1 μmol/L) was added. After 30 minutes of reequilibration periods, myogenic responses were again assessed. Finally, high-potassium medium was added. Previous results suggest that in our experimental model, staurosporine (50 mmol/L) and thapsigargin (1 μmol/L) blocked the activation of protein kinase C and calcium mobilization, respectively.

All experimental procedures were approved by our institutional ethical committees. Data were expressed as mean ± SE. Statistical significance was examined by linear regression analysis, Student’s t test, and ANOVA followed by the Newman-Keuls test. P < 0.05 was considered significant.

Results

Increasing pressure constricted all segments of ILAs. As shown in Figure 2, the elevation of renal arterial pressure (RAP) from 80 to 120 mm Hg decreased the diameter of distal ILAs by 2.2 ± 0.6 μm (n = 5). Distal ILAs were further constricted at RAP of 160 mm Hg (−5.0 ± 0.7 μm). Similarly, intermediate ILAs manifested decrements in their diameters in response to increasing RAP from 80 to 120 mm Hg (−2.7 ± 1.1 μm; n = 5). At RAP of 160 mm Hg, the diameter of intermediate ILAs was reduced by 5.1 ± 0.8 μm. In contrast, although the elevation of RAP from 80 to 120 mm Hg did not contract proximal ILAs, further pressure increments to 160 mm Hg elicited significant myogenic constriction (−3.3 ± 1.2 μm; n = 5). Figure 3 describes the magnitude of myogenic responsiveness plotted against basal ILA diameters at 80 mm Hg. There was a linear relationship between 2 parameters. ILAs with smaller diameters manifested a greater degree of myogenic constriction.

Although administration of diltiazem (10 μmol/L) failed to alter basal diameters in all segments of ILAs at 80 mm Hg, it inhibited myogenic responses of ILAs (Figure 2). In distal ILAs, diltiazem completely abolished myogenic constriction. The elevation of RAP from 80 to 160 mm Hg did not alter distal ILA diameter (0.0 ± 0.6 μm; P < 0.01 versus control response). Diltiazem also prevented myogenic constriction of intermediate ILAs. In the presence of diltiazem, pressure stimuli of 160 mm Hg did not decrease intermediate ILA diameters (−1.1 ± 0.7 μm; P < 0.01 versus control). In addition, diltiazem blocked myogenic constriction of proximal
ILAs. At RAP of 160 mm Hg, proximal ILA diameter was maintained (21.3 ± 0.8 mm; P, 0.05 versus control). Subsequent increase in potassium concentration did not alter distal (from 29.1 ± 3.0 to 28.9 ± 3.3 mm), intermediate (from 49.7 ± 3.0 to 49.0 ± 3.5 mm), or proximal ILA diameters (from 76.4 ± 5.9 to 75.8 ± 6.0 mm), suggesting sufficient inhibition of L-type voltage-dependent calcium channels by this dose of diltiazem.

Figure 4 depicts the effects of gadolinium and NCDC on ILA myogenic responses. Under control conditions, myogenic responses were well preserved in all segments of ILAs. Thus, increasing RAP from 80 to 160 mm Hg elicited 17±2%, 10±2%, and 5±1% decrements of diameters in distal (−4.8±0.8 μm; n=6), intermediate (−4.4±0.6 μm; n=6), and proximal ILAs (−3.8±1.0 μm; n=6), respectively. Gadolinium abolished myogenic constriction of distal ILAs. In the presence of gadolinium, the elevation of RAP from 80 to 160 mm Hg did not alter distal ILA diameter (−1.0±1.1 μm; P<0.01 versus control). In contrast, myogenic constriction of intermediate ILAs was partially attenuated by gadolinium. Pressure stimuli of 160 mm Hg significantly constricted intermediate ILAs by 2.2±0.7 μm (P<0.05 versus control). Finally, gadolinium failed to alter myogenic responsiveness of proximal ILAs. Increasing RAP from 80 to 160 mm Hg decreased the diameter of proximal ILAs by 3.1±0.6 μm (P=NS versus control). NCDC increased basal diameter of proximal ILAs from 79.3±5.2 to 85.8±6.2 μm (P<0.05). Furthermore, myogenic contraction of proximal ILAs was prevented by the addition of NCDC (0.0±0.8 μm [at 160 mm Hg]; P<0.05 versus control).

Figure 3. Relationship between myogenic responsiveness and basal diameter of ILA at 80 mm Hg. A greater degree of myogenic constriction (80 to 160 mm Hg) was observed in the ILA with smaller basal diameter (α=0.28±0.03%/μm; n=15; P<0.01 versus zero).

Figure 4. Influence of gadolinium and NCDC on myogenic responses in ILA. Gadolinium (■) blocked myogenic constriction in distal ILAs. Subsequent addition of NCDC (●) inhibited myogenic contraction in intermediate and proximal ILAs. Controls are also shown (○). *Significant difference from respective basal value at 80 mm Hg.

Figure 5. Relationship between inhibitory effects of gadolinium on myogenic responsiveness and basal diameter of ILA at 80 mm Hg. Stronger inhibition of myogenic constriction (80 to 160 mm Hg) was observed in the ILA with smaller basal diameter (α=−1.4±0.2%/μm; n=18; P<0.01 versus zero).

Figure 5. Relationship between inhibitory effects of gadolinium on myogenic responsiveness and basal diameter of ILA at 80 mm Hg. Stronger inhibition of myogenic constriction (80 to 160 mm Hg) was observed in the ILA with smaller basal diameter (α=−1.4±0.2%/μm; n=18; P<0.01 versus zero).
Subsequent addition of high-potassium media reduced distal, intermediate, and proximal ILAs by 34±5%, 20±3%, and 7±2%, respectively (P<0.01). These data suggest that the doses used, gadolinium or NCDC does not interact nonspecifically with voltage-dependent calcium channels.

Since several lines of evidence indicate that phospholipase C could be activated through gadolinium-sensitive mechanisms,20 direct influences of NCDC on ILA myogenic responses were assessed. Similar to the previous groups, all segments of ILAs responded to pressure stimuli under control conditions (Figure 6). Administration of NCDC altered basal diameter of neither distal (from 26.4±2.8 to 26.6±2.6 μm; n=6) nor intermediate ILAs (from 49.4±2.8 to 50.7±2.9 μm; n=6) at RAP of 80 mm Hg. Furthermore, NCDC did not alter myogenic responsiveness of distal ILAs. In the presence of NCDC, increasing RAP from 80 to 160 mm Hg constricted distal ILAs (−3.9±1.2 μm; P=NS versus control). However, the addition of NCDC attenuated myogenic constriction of intermediate ILAs. When treated with NCDC, the elevation of RAP from 80 to 120 mm Hg did not elicit constriction in intermediate ILAs, and the diameter of intermediate ILAs was decreased only by 3.4±1.0 μm at RAP of 160 mm Hg (P<0.05 versus control [−5.4±0.9 μm]). In proximal ILAs, NCDC abolished myogenic constriction. Basal diameter of proximal ILAs at 80 mm Hg was increased by NCDC (from 75.2±4.3 to 78.3±4.5 μm; n=6; P<0.05). Furthermore, alterations in RAP from 80 to 160 mm Hg failed to alter proximal ILA diameters (−0.2±1.1 μm; P=0.05 versus control).

Figure 7 describes the effects of staurosporine and thapsigargin on ILA myogenic responses. Under control conditions, all segments of ILAs responded to increasing RAP. In distal ILAs, neither staurosporine nor thapsigargin altered basal diameter (from 25.6±3.3 to 26.0±3.5 μm; n=6) and myogenic responsiveness, suggesting that these blockers possess little effect on mechanosensitive cation channels and myosin light chain kinase. In intermediate ILAs, myogenic responsiveness was not altered by the administration of staurosporine but was attenuated with thapsigargin. In the presence of thapsigargin, the elevation of pressure from 80 to 160 mm Hg elicited only 6±2% reductions in intermediate ILA diameters (−2.9±1.0 μm; n=6; P<0.05 versus control [−5.1±1.0 μm]). In proximal ILAs, treatment with staurosporine increased their diameters at RAP of 80 mm Hg (from 77.8±5.3 to 80.9±5.5 μm; n=6; P<0.05). However, in the presence of staurosporine, increasing pressure contracted proximal ILAs (−4.5±0.8 μm [at 160 mm Hg]; P=NS versus control). Subsequent addition of thapsigargin virtually abolished myogenic constriction of proximal ILAs. Under the blockade of both protein kinase C and calcium mobilization, raising pressure from 80 to 160 mm Hg did not elicit proximal ILA contraction (−1.5±1.1 μm; P<0.01 versus control). High-potassium media decreased distal (from 25.7±3.3 to 15.2±3.1 μm; P<0.01), intermediate (from 50.6±3.3 to 38.5±3.5 μm; P<0.01), and proximal ILA diameters (from 81.8±5.5 to 72.1±5.6 μm; P<0.01).

Discussion

ILAs arise from arcuate arteries near the corticomedullary junction and terminate at afferent arterioles in the cortex.21 ILAs taper along their length, varying in diameter from >90 μm near the arcuate artery to <30 μm at the terminal segment. Thus, ILAs constitute important resistance vessels relating to renal autoregulation. Indeed, intraluminal pressure within proximal ILAs near the arcuate artery is directly related to systemic blood pressure,11 whereas that within distal ILAs at the terminal segment is partially autoregulated.10 In accordance, recent investigations using various approaches demonstrated that increasing RAP elicited ILA constriction.5,8,18,22 However, the mechanisms mediating autoregulatory responses of ILAs were not fully characterized. Furthermore, differing mechanisms may underlie myogenic responses in distinct segments of ILAs.

ILAs appear to possess segmental differences in the magnitude of increase in vascular resistance responding to elevating pressure. Since vascular resistance is related to 1/radius4 (Hagen-Poiseuille equation), vessels with smaller diameters should provide greater vascular resistance. Furthermore, our results are consistent with observations that vessels with smaller calibers manifest stronger myogenic responses1 and indicate that ILAs with smaller diameters manifested greater degree of myogenic constriction (Figure 3). Thus, the
present data support the previous findings that intravascular pressure in distal ILAs was partially autoregulated and further suggest that myogenic constriction of ILAs, especially distal segments, substantively participates in total renal vascular resistance adjustments responding to pressure.

In the present study we demonstrated that myogenic constriction in all segments of ILAs was prevented by diltiazem. The present results support previous observations by Casellas and Bouriquet that nimodipine inhibited myogenic constriction of ILAs and arcuate arteries. Furthermore, we showed that thapsigargin did not alter myogenic constriction of distal ILAs. The observations that high-potassium media constricted ILAs in the presence of thapsigargin suggest that calcium entry through L-type calcium channels increases cytosolic calcium sufficient to induce ILA constriction. Our findings, however, differ from those of Inscho et al that thapsigargin inhibited pressure-induced afferent arteriolar constriction. Because the afferent arteriole is the target of TGF and because calcium mobilization plays an important role in transducing TGF, the variance in observed sites may account for the discrepancy. Calcium antagonists nullify TGF signals at the effector level. Finally, recent results demonstrated that the autoregulation of total renal blood flow was virtually abolished by the blockade of voltage-dependent calcium channels. Collectively, these results indicate that as afferent arterioles, the activation of voltage-dependent calcium channels mediates ILA myogenic constriction, and they suggest that L-type calcium channels play a crucial role in autoregulatory adjustments of renal vascular resistance.

Although there seems to be a consensus on a mediatory role of voltage-dependent calcium channels in renal autoregulation, the ionic mechanisms mediating activation of L-type calcium channels by pressure are poorly understood. We have previously provided evidence that pressure-induced activation of mechanosensitive cation channels, a membrane-delimited process, underlies afferent arteriolar myogenic constriction. Although gadolinium may interact with L-type calcium channels, which are essential for myogenic constriction in distal ILAs, high-potassium media constricted distal ILAs in the presence of gadolinium. Furthermore, our data indicated that proximal ILA myogenic constriction was inhibited by diltiazem but not gadolinium. In addition, while calcium entry through gadolinium-sensitive channels may stimulate phospholipase C, our demonstrations that NCDC itself did not significantly alter distal ILA myogenic constriction mitigate against a dominant role of phospholipase C in distal ILA myogenic constriction. Thus, these findings suggest that in a manner similar to that of afferent arterioles, pressure stimuli increase the open probability of mechanosensitive cation channels on distal ILAs, thereby eliciting membrane depolarization and activation of voltage-dependent calcium channels.

In contrast to distal ILAs, gadolinium exhibited little inhibitory effect on myogenic constriction of proximal ILAs, suggesting a small role of membrane-delimited mechanism in this response. Roman and Harder have demonstrated that increasing pressure raises both inositol trisphosphate and diacylglycerol in renal arteries. They suggest that in a manner similar to that of aortic myocytes, pressure stimuli activate phospholipase C in renal arteries, thereby mediating myogenic constriction. In agreement, we showed that NCDC, a phospholipase C inhibitor, abolished myogenic constriction of proximal ILAs. Although the addition of either NCDC or staurosporine increased proximal ILA diameters at RAP of 80 mm Hg, myogenic constriction was blocked only by NCDC, validating that ILA dilation itself does not impair myogenic constriction. The present data provide evidence that phospholipase C mediates myogenic constriction of proximal ILAs and suggest that at the RAP of 80 mm Hg, pressure-induced activation of phospholipase C is already operative in proximal ILAs.

Since phospholipase C produces 2 important second messengers, we assessed the effects of staurosporine, a protein kinase C blocker, and thapsigargin, which depletes calcium store, on myogenic responses. Osol et al suggest the participation of protein kinase C in the development of basal tone. Similarly, we have shown that the administration of staurosporine elicited vasodilation of proximal ILAs at RAP of 80 mm Hg. Ample evidence indicates that protein kinase C enhances sensitivity of contractile elements to a given concentration of cytosolic calcium. Indeed, our previous data suggest that endothelin-induced activation of protein kinase C increases the sensitivity of contractile elements to calcium in preglomerular microvasculature. Collectively, these data suggest that protein kinase C constitutes a determinant of basal tone in proximal ILAs. On the other hand, Roman and Harder suggested that pressure induces intracellular calcium mobilization in vascular smooth muscle. The present observations that the treatment with thapsigargin blocked myogenic constriction of proximal ILAs are compatible with previous findings and suggest a pivotal role of calcium mobilization in activating voltage-dependent calcium channels on this ILA segment by pressure. Elevation of cytosolic calcium could elicit membrane depolarization directly by opening chloride channels on proximal ILAs and/or indirectly by closing potassium channels through the stimulation of phospholipase A.

Intermediate ILAs appear to exhibit characteristics between those of distal and proximal ILAs. In the present study we have demonstrated that either gadolinium or NCDC attenuated but did not block myogenic constriction of intermediate ILAs. However, simultaneous treatment with both gadolinium and NCDC abolished intermediate ILA myogenic constriction. Our data provide evidence that both membrane-delimited and phospholipase C–dependent processes are required for full activation of voltage-dependent calcium channels in intermediate ILAs during myogenic stimulation. Since gadolinium exhibited greater inhibitory effects on myogenic constriction in ILAs with smaller diameter (Figure 5), gadolinium-sensitive mechanosensitive cation channels may prevail in small ILAs. Taken together, these findings suggest that intermediate ILAs constitute transitional sites regarding the mechanisms underlying myogenic responses.

A caveat should be considered. Although calcium entry through mechanosensitive cation channels may trigger phospholipase C, our data demonstrated that gadolinium failed to induce substantial alterations in myogenic constriction of proximal ILAs. Kulik et al suggested that physical stimuli
stimulate phospholipase C through the pathways insensitive to pertussis toxin. As reviewed recently, Gq proteins may be involved in triggering phospholipase C during myogenic activation. Clearly, further studies are required to elucidate precise mechanisms of the activation of phospholipase C by pressure stimuli.

In summary, the present data indicate that mechanisms mediating myogenic responses differ among distinct segments of ILAs. In addition, our results suggest that increasing pressure gates mechanosensitive cation channels, thereby eliciting membrane depolarization and activation of voltage-dependent calcium channels in distal ILAs. Finally, our findings provide evidence that pressure-induced stimulation of phospholipase C mediates myogenic contraction of proximal ILAs.

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