An Arg-Gly-Asp peptide stimulates constriction in rat afferent arteriole

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Yip, Kay-Pong, and Donald J. Marsh. An Arg-Gly-Asp peptide stimulates constriction in rat afferent arteriole. Am. J. Physiol. 273 (Renal Physiol. 42): F768–F776, 1997.—The potential role of integrins in the myogenic mechanism was studied in the rat afferent arteriole (AA) by fluorescence immunolocalization and micropenetration of isolated AAs. Confocal fluorescence images were acquired from frozen sections of rat kidney after indirect immunostaining for various integrin β- and α-subunits. The β3, β5, α5, and αv-integrins were found on the plasma membrane in smooth muscle of AA, providing the morphological basis for participation of integrins in mechanotransduction. With 1 mM nitro-L-arginine methyl ester (L-NAME) in the luminal perfusate to inhibit endogenous nitric oxide (NO) production from AA, the hexapeptide GRGDSP (10⁻² to 10⁻³ M) induced immediate vasoconstriction. The constriction was dose dependent and specific for peptides with arginine-glycine-aspartic acid (RGD) motifs, commonly found on the binding sites of extracellular matrix to integrins. In controls, the hexapeptide GRGESP induced no constriction. GRGDSP, 1 mM, induced a 21.6 ± 2.6% decrease in lumen to 60 s and an 18.3 ± 4.1% increase in lumen to 60 s in smooth muscle intracellular calcium concentration for 18 s, as measured by the emission ratio of Fluo-3/Fura Red. Binding of exogenous RGD motifs with exposed integrins on AA smooth muscle therefore triggers calcium-dependent vasoconstriction. However, the dose response to RGD was not sensitive to the myogenic tone of the vessel, which suggests that the integrin-mediated vasoconstriction is different from myogenic constriction.

confocal fluorescence microscopy; mechanotransduction; intracellular calcium

A MYOGENIC RESPONSE and tubuloglomerular feedback (TGF) are the two major mechanisms responsible for renal blood flow autoregulation. The sensor responsible for the role of TGF in regulating afferent arteriolar (AA) resistance is known (2), but the signal transduction in myogenic response in AA is not elucidated. Studies of myogenic mechanisms from other vascular beds suggest that an increase of transmural pressure activates stretch-sensitive cation channels in vascular smooth muscle cells (VSMC), which open voltage-gated calcium channels by depolarization (9, 16). The influx of extracellular Ca²⁺, plus the release from endogenous Ca²⁺ stores, triggers the initial myogenic constriction (8). Stretch-sensitive mechanisms can account for the trigger of myogenic constriction. However, the stretch resulting from an increase of transmural pressure will diminish once the vessel contracts. The signal for maintaining the myogenic constriction after the initial response to stretch is not identified.

The transmembrane heterodimeric proteins, integrins, have been shown to mediate the transduction of mechanical force from an extracellular site into the cell via their cytoplasmic connections with the cytoskeleton (30). Evidence of integrin involvement in stretch-related phenomena has been reported in the motor nerve terminal. Chen and Grinnell (4) have found that stretch-enhanced release of neurotransmitter from the frog motor terminal is calcium dependent and can be inhibited by either integrin antibodies or peptides containing the sequence arginine-glycine-aspartic acid (RGD). RGD is a common motif found in many extracellular matrix proteins that bind to integrins. Stretch resulting from changes in the transmural pressure of arterioles will alter the attachment between the extracellular matrix and the integrins of vascular smooth muscle. Exogenous RGD peptides have been found to alter smooth muscle intracellular Ca²⁺ concentrations ([Ca²⁺]) in skeletal arterioles (7). Soluble RGD peptide inhibited the activity of L-type calcium channel in isolated smooth muscle cells, whereas fibronectin-coated beads increased the Ca²⁺ current (32). [Ca²⁺] in smooth muscle is known to be the primary determinant of myogenic stimulation (16, 33). These observations suggested that changes in the interactions between integrins and extracellular matrix due to stretch might be involved in myogenic response by altering smooth muscle [Ca²⁺].

If integrins are involved in stretch-related signal transduction in rat AA, then integrins should be localized in the plasma membrane of smooth muscle. Binding of exogenous RGD to integrins on smooth muscle might alter the arteriole’s contractile state, probably by altering smooth muscle [Ca²⁺]. The effects of exogenous RGD on the contractile state arteriole response should be sensitive to the myogenic tone of the vessel. In a relaxed state, exogenous RGD peptide might cause contraction of vascular smooth muscle, but in a contracted state as would be present with increased transmural pressure gradient, RGD peptide might cause relaxation (17). The goals of the present study were therefore 1) to test for the presence of integrins on the vascular smooth muscle of AA with indirect immunofluorescence microscopy, 2) to test whether exogenous peptides containing the RGD sequence can alter the contractile state of AA and whether this response is dependent on the underlying myogenic tone, and 3) to investigate whether the exogenous RGD sequence-containing peptides can induce changes in smooth muscle [Ca²⁺] in AA.

MATERIALS AND METHODS

Tissue preparation for immunofluorescence. Sections of kidney for immunostaining were obtained from male Sprague-Dawley rats (180–250 g, Harlan). Rats were anesthetized...
with halothane. Kidneys were perfusion fixed with a mixture of 4% paraformaldehyde and 4% sucrose in phosphate-buffered saline (PBS) by retrograde perfusion via a cannula inserted into the descending aorta distal to the renal artery. The kidneys were then removed, and blocks of tissue were cut into 4-mm cubes. Blocks of tissue were postfixed overnight in the same fixative at 4°C, washed with 0.1 M aqueous NH$_4$Cl solution for 30 min, and cryoprotected by incubation in 2.3 M sucrose in PBS for 1 h. The tissue blocks were then snap frozen in optimum cutting temperature compound (OCT, Miles Laboratory) with liquid nitrogen-cooled isopentane. Cryosections (7 µm) were cut and transferred to Fisher Superfrost Plus-charged glass slides. Sections were first incubated with 1% sodium dodecyl sulfate (SDS) in PBS for 5 min for antigen retrieval as suggested by Brown et al. (3). After SDS was removed by washing in PBS, the sections were blocked with 20% donkey serum in PBS for 20 min and then incubated with a mixture of anti-smooth muscle α-actin antibodies and antibodies specific to each integrin subunit for double labeling. After 2-h incubation, the sections were washed three times with fresh PBS followed by incubation with the appropriate CY5-conjugated secondary antibodies for 60 min. All incubations were carried out in a moistened chamber at room temperature. The sections were then rinsed with fresh PBS three times, mounted in glycerol, and examined with a ×63 plan-apochromat objective (NA 1.4, oil immersion) on a Zeiss Axiovert model 100TV inverted microscope. Glycerol is used as a mounting medium to minimize the effects of refractive index mismatch during the confocal image acquisition, which will cause significant aberration in three-dimensional image reconstruction or merging of images in multiple-labeling studies if not corrected (12, 14). The microscope is coupled to a model MRC-1000 confocal scanning unit (Bio-Rad) equipped with a krypton-argon laser, three photomultiplier detectors, and one transmitted light detector. Frozen sections stained with only the secondary antibody were used as negative controls.

Antibodies. Rabbit polyclonal anti-rat β$_3$ antibody was kindly provided by Dr. S. Adler (1). Rabbit polyclonal α$_3$ and α$_5$ antibodies were purchased from Chemicon (Temecula, CA), and mouse monoclonal immunoglobulin G (IgG) antibody to α$_5$ was purchased from Sigma Chemical Co. (St. Louis, MO). Mouse monoclonal IgM antibody to β$_3$ was purchased from Transduction Laboratories (Lexington, KY). The anti-β$_3$ antibody can recognize the extracellular domain of integrin, whereas the others recognize the cytoplasmic domain of integrins. Fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal IgG to smooth muscle α-actin was purchased from Sigma Chemical (St. Louis, MO). Dilution for all primary antibodies was determined prior to the experiment. The primary antibodies for integrins were selectively purchased from different vendors to optimize their specificity. All secondary conjugated antibodies were affinity purified and were designed for multiple labeling study. Secondary antibodies were diluted at 1:400 for all studies (Jackson Immunoresearch Laboratory, West Grove, PA).

Microperfusion of AA. Experiments were conducted in AA isolated from rat juxtamedullary nephrons as reported previously (33). In brief, a segment of afferent arteriole (300-400 µm) just proximal to a glomerulus was dissected, cannulated, and perfused in a temperature-controlled perfusion chamber (Vestavia, AL) mounted on a Zeiss Axiovert 100TV inverted microscope. The intraluminal pressure of the vessel was initially set at 80 mmHg. Vessels were discarded if there was fluid leakage. The effects of the peptides GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro), cyclic GRGDSP (cyclic Gly-Pen-Gly-Arg-Gly-Asp-Ser-Pro-Cys-Ala), and GRGESP (Gly-Arg-Gly-Gr
brush border of proximal tubules (Fig. 1C). The signals of \( \beta_2 \) on glomeruli and AA were weaker than those of \( \beta_1 \). Three other \( \alpha \)-integrin subunits, \( \alpha_3 \), \( \alpha_5 \), and \( \alpha_{V} \), were also localized on the basolateral plasma membrane of AA smooth muscle cells. The strongest signal of \( \alpha_3 \) was found on the glomeruli (Fig. 2a). A strong signal of \( \alpha_5 \) was found on the thick ascending limbs (Fig. 2C), whereas the signal from the AA was barely visible. The strongest signal of \( \alpha_V \) was recognized in thick ascending limbs (Fig. 3). These observations confirmed the presence of diversified integrins in the smooth muscle of AA, which could provide the morphological basis for integrin to participate in myogenic response. Negative controls, made by omitting the primary antibodies in the staining procedures, demonstrated no detectable signal in arterioles. The control indicates successful blocking as well as the specificity of the secondary antibodies. The strong background in the localization of autofluorescence from proximal tubules was due to the autofluorescence from proximal tubules. The intensity of autofluorescence in proximal tubules is wavelength dependent. Autofluorescence from proximal tubules was minimal when fluorescence was collected with CY5 filter set (excitation 647 nm) compared to FITC filter set (excitation 488 nm). To demonstrate the abundance of integrins on the plasma membrane of smooth muscle cells and on the endothelial cells, which could not be revealed by immunofluorescence on cryosections, immunolocalization of integrins was also performed in a few isolated AA with anti-\( \beta_3 \) antibody. This antibody can recognize the extracellular domain of \( \beta_3 \)-integrin. Figure 4, A and B, shows confocal fluorescence micrographs of integrin \( \beta_3 \)-subunit on AA. Spindle-shaped smooth muscle cells with immunofluorescence on the plasma membrane could be recognized (Fig. 4A). The exposed endothelium was heavily stained by the integrin antibodies (Fig. 4B).

Effects of RGD-containing peptides in perfused AA. There was no consistent significant change of lumen diameter in AA when GRGDSP (10^{-5}–10^{-3} M) was introduced into the perfusion chamber (data not shown). This observation suggested that either the exogenous GRGDSP peptides had no effect on the contraction of AA or the effects induced by the exogenous RGD peptide were masked by some other mechanism. One possibility is that the continuous accumulation of endogenous NO from AA endothelium (20) masks the constriction induced by the GRGDSP peptide. To test this hypothesis, 1 mM l-NAME was included in the luminal perfusate to inhibit the NO synthase before the GRGDSP was introduced into perfusion chamber. Impairment of NO production by l-NAME was suggested...
by a reduced lumen diameter and the ability of 1 mM L-arginine to reverse the constriction. The mean diameters of AA before and after NO synthase blockade were 22.3 ± 1.6 and 16.3 ± 1.4 µm (P < 0.05, n = 12), respectively. With 1 mM L-NAME in the luminal perfusate, addition of GRGDSP to the bathing solution elicited a persistent vasoconstriction in AA. The constriction was dependent on the dose of GRGDSP over a range of concentrations from $10^{-7}$ to $10^{-3}$ M (Fig. 5). The peptide GRGESP, which does not carry the RGD motif, failed to elicit constriction (Fig. 5), indicating that constriction was specific to the RGD motif. Another RGD peptide, cyclic GRGDSP, was used to test whether the dose-dependent constriction was sensitive
to the steric conformation of the RGD motif. The dose-response curves of these two RGD-containing peptides were similar (Fig. 5), suggesting that the induced constriction was not sensitive to the steric conformation of the RGD motif.

The average time course of variations in lumen diameters of AA induced by 1 mM GRGDSP is shown in Fig. 6. The mean basal diameter was 16.6 ± 2.1 µm (n = 8). The constriction was immediate and reached a maximum within 6 s and remained significantly constricted for another 35 s (P < 0.05). The original vessel diameter was fully restored after the removal of the peptides by washing the perfusion chamber. The average constriction was 19.7 ± 2.1% (P < 0.05, n = 8) of the baseline diameter in the first 30 s after the application of the peptide. To determine whether the constriction triggered by the RGD sequence-containing peptides is associated with a rise in VSMC [Ca2+]i, the simultaneous variations of luminal diameter and VSMC [Ca2+]i in the perfused vessels were determined in a separate study. Addition of 1 mM GRGDSP to the bath triggered an immediate constriction in the vessel as observed previously (Fig. 7B). Constriction was completed after 5 s, and the average constriction for the first 30 s was 21.6 ± 2.6% (P < 0.05, n = 6) of the baseline. The emission ratio of Fluo-3/Fura Red, a ratiometric index of the relative change of VSMC [Ca2+]i, increased immediately after the addition of 1 mM GRGDSP to the bath (Fig. 7A). The emission ratio reached its peak value after 5 s of RGD peptide application and remained elevated for 18 s. Both the increase in emission ratio and decrease in lumen...
myogenic constriction (15.6 ± 0.5 µm (n = 6)). Mean diameter in the baseline is 18.1 ± 0.3 µm (n = 6).

Fig. 7. Normalized time course of changes in AA smooth muscle [Ca^{2+}] (A) and the corresponding change in lumen diameter when the vessels were exposed to 1 mM GRGDSP (B). RGD peptide was added to the perfusion chamber at time 0. Dotted lines are SE. Solid circles (●) indicate that the contraction is significant (P < 0.05, n = 6). Mean diameter in the baseline is 18.1 ± 0.3 µm (n = 6).

diameter are significantly different (P < 0.05) from the baseline 2 s after the peptide application. These observations indicate that the exogenous RGD-containing peptide triggers the constriction by elevating smooth muscle [Ca^{2+}].

To test the effect of myogenic tone of AA on the GRGDSP-induced vasoconstriction, the responses of the luminal diameter to variations in dose were determined at perfusion pressures of 80, 120, and 160 mmHg, respectively, in a separate study. Significant myogenic constriction (P < 0.05, n = 5, Table 1) was found when perfusion pressure was increased. However, there was no significant difference in the constriction induced by GRGDSP when the myogenic tone of the vessels was increased (Table 1). The myogenic constriction and GRGDSP-induced constriction were additive. If integrins were involved in the myogenic mechanism, then there should have been an effect of underlying vascular tone on the response to exogenous RGD. No such interaction was observed. These observations suggested that RGD-induced constriction was not related to myogenic constriction, despite the fact that exogenous RGD-containing peptides could induce vasoconstriction via an elevation of smooth muscle [Ca^{2+}].

DISCUSSION

There is only a limited amount of literature available regarding the distribution of integrins in the afferent arterioles (1). To pursue the hypothesis that integrins might be involved in regulating the tone in AA, indirect immunofluorescence microscopy was employed to establish their presence in the AA as well as their distribution along the nephron. The β₁- and α₁-integrins were found on the basolateral plasma membrane of smooth muscle cell in AA. The apical immunofluorescence signal from AA was most likely the result of integrins on the endothelial cells rather than on smooth muscle cells, because the immunofluorescence signal did not colocalize with the smooth muscle α-actin and the endothelium of isolated AA was positive labeled by integrin antibodies. These observations were also consistent with the report of identification of RGD peptide binding sites on endothelium of renal vasculature (23).

The α- and β-integrin subunits found in AA are similar to those found in rat tail and mesenteric arteries (29), except that no α₃ was found in the latter two vessels. Because the availability of antibodies for immunolocalization was limited by species specificity, it is possible that other integrin subunits, which have not been tested, might also be present in the smooth muscle of AA. Two of these untested integrins are β₄ and α₆, which have been reported in human resistance vessels (6). The β₁- and β₂-integrins are the two most widely expressed β-subunits through which the extracellular matrix is connected to cytoskeleton (5). Although not all β- and α-integrin subunits in the AA have been identified, the presence of β₁ and β₂ in AA is sufficient to provide the morphological basis required to transmit a mechanical signal from the extracellular matrix into the cytosol of smooth muscle, which could be an integral part of myogenic response in AA.

Table 1. Effects of perfusion pressure and exogenous GRGDSP on the normalized luminal diameter

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<tr>
<th>GRGDSP, mM</th>
<th>Perfusion Pressure</th>
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<tr>
<td>0.0</td>
<td>1.00 ± 0.02</td>
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<tr>
<td>0.1</td>
<td>0.81 ± 0.04</td>
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<tr>
<td>1.0</td>
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Values are means ± SE. Dose response was examined in each vessel at 3 different perfusion pressures. Baseline diameter at 80 mmHg of each vessel was used to normalize changes in diameter in the same vessel. Mean baseline diameter with a perfusion pressure of 80 mmHg was 15.6 ± 1.8 µm (n = 5). Myogenic constriction at 120 and 160 mmHg is significant (P < 0.05) compared with 80 mmHg. Induced constriction by 0.1 and 1 mM of the hexapeptide GRGDSP is significant (P < 0.05) compared with the control at the same perfusion pressure.
The next hypothesis tested was whether exogenous RGD sequence-containing peptides could alter the vessel’s tone. If integrins were involved in regulating the smooth muscle tone, then binding of exogenous RGD-containing peptide to the exposed integrins in smooth muscle cells might alter the contractile state of AA. In the presence of L-NAME to inhibit endothelial NO production, exogenous GRGDSP peptides induced a dose-dependent constriction in perfused AA. NO is known to be released continuously from endothelium to modulate the tone of renal arterioles (20). The accumulation of NO is particularly significant in perfused arterioles, because the continuous release of NO is unopposed by the NO scavenging property of hemoglobin (33). The requirement for NO synthase blockade to unmask constriction of RGD peptide need not imply interactions between integrins and NO. GRGDSP in the micromolar range induced the shortening of freshly isolated renal VSMC in the absence of L-NAME (unpublished observation). Since the control peptide, GRGESP, failed to induce constriction at any dosage tested, the constriction was specific for peptides with RGD motifs. These observations are consistent with the hypothesis that binding of integrins on smooth muscle with exogenous RGD peptides could alter the vascular tone.

The binding of exogenous RGD peptides to some heterodimers of integrins (for example, α2β1) is known to be sensitive to the steric conformation of the RGD motif (17, 18). The possibility that the RGD-induced constriction was sensitive to the steric constraint of the RGD motif was tested by constructing the dose-response curve using a cyclic GRGDSP and comparing the dose-response curve to that of its linear counterpart, GRGDSP. However, there was no significant difference between the dose-response curves of these two peptides. These observations indicated that the integrin-mediated constriction in AA was not sensitive to the steric constraint of the RGD motif. Interestingly, Mogford et al. (17) showed that cyclic GRGDSP is more powerful than the linear GRGDSP in changing the vascular tone of arterioles isolated from rat cremaster muscle. The α2β1-integrin was suggested to be the binding site for the cyclic GRGDSP in this preparation (17).

The time course of change in lumen diameter showed that the constriction induced by the RGD was rapid and reversible. The onset of constriction was almost immediate and reached a maximum after 6 s. The vessels started to recover toward the baseline after 35 s, but full recovery was achieved only when the RGD peptides were removed from the perfusion chamber. These findings indicated that either the signal generated by the exogenous RGD motifs was transient in nature or that the vessel was desensitized very rapidly to that signal. However, repeated applications of RGD peptides induced similar vasoconstrictions (data not shown), showing no sign of desensitization. The signal generated by the binding of exogenous RGD to the integrins in AA was probably diminished once the vessel was contracted. Contraction of the vessel would reduce the surface area exposed to the exogenous RGD, thereby reducing the ratio of integrins bound by exogenous RGD to the extracellular matrix and thus the impact of exogenous RGD on the vessel’s tone.

[Ca2+]i-dependent activation of myosin light chain kinase and its phosphorylation of the 20-kDa light chain of myosin is generally considered the primary mechanism responsible for regulation of contractile force in arterial smooth muscle (22). One possible mechanism by which RGD peptide induces vasoconstriction is by elevating the [Ca2+]i, of AA smooth muscle. This hypothesis was tested by simultaneously monitoring the smooth muscle [Ca2+]i, and vessel diameter by using confocal fluorescence microscopy coupled to digital image processing technique. Collecting the fluorescence image of the vessel confocally enabled us to measure the lumen diameter simultaneously with smooth muscle [Ca2+]i, without the complication of out-focus fluorescence, e.g., fluorescence arising from the endothelium (33). Smooth muscle [Ca2+]i was measured by the emission ratio of Fluo-3/Fura Red. The emission ratio is independent of the changes of local dye concentration that occur during the constriction of the vessel (13, 33). Exposure of the perfused AA to RGD peptides induced an immediate increase of smooth muscle [Ca2+]i, which then decreased gradually after 18 s. The time course of change in smooth muscle [Ca2+]i coincided for the most part with the change of lumen diameter. The initially elevated smooth muscle [Ca2+]i was restored toward the baseline after 18 s, whereas the vessel diameter became not significantly different from the baseline after 30 s. The relaxation in AA was delayed compared to the smooth muscle [Ca2+]i profile. This delay might reflect the latch phenomenon in stimulated smooth muscle or increased sensitivity of calcium-dependent phosphorylation in smooth muscle (11, 22).

The mechanism of the RGD-induced increase of smooth muscle [Ca2+]i is not addressed in this study. In cremaster arteriole smooth muscle, RGD peptides inhibited the Ca2+ current, whereas fibronectin-coated beads increased the Ca2+ current (32). However, RGD peptides induced dilatation in cremaster muscle arteriole (17) but constriction in AA. The mechanisms of altering smooth muscle [Ca2+]i might be different in these two vascular beds. Elevation of [Ca2+]i in integrin-mediated attachment between cultured cells and their substrate was well documented (21, 25–27, 34). In Madin-Darby canine kidney cells, binding with RGD peptide-coated beads increases the Ca2+ current (32). Integrin antibodies immobilized on surface can also increase [Ca2+]i in endothelial cells (24). The transient increase of [Ca2+]i in smooth muscles cells induced by RGD-containing peptides is consistent with these observations. There is no consensus on how the occupancy and clustering of integrins could elevate the [Ca2+]i in these cells. Mobilization of intracellular calcium stores was suggested in rat osteoclasts (34), whereas the voltage-independent calcium channel was implicated in human endothelial cells (24).

Myogenic constriction of AA is associated with an sustained increase in smooth muscle [Ca2+]i (33).
GRGDSP-induced constriction is also associated with increased [Ca^{2+}]. If the signaling mechanisms of these two constrictions have certain elements in common, then activation of myogenic constriction might alter the response induced by GRGDSP. One potential common element is the interactions of integrin with RGD motifs from extracellular matrix and GRGDSP peptides. However, the dose response of GRGDSP seemed to be insensitive to the myogenic tone of the vessel. The effects of transmural pressure and RGD peptide were additive, which suggested that the RGD-induced vasoconstriction was not related to myogenic constriction. An alternative explanation was that the myogenic constriction and RGD-induced vasoconstriction were mediated by two separate populations of integrins.

Although RGD peptides induce vasoconstriction by increasing the smooth muscle [Ca^{2+}], in renal arterioles, the effects seemed to be vascular bed specific. Fragments of collagen and RGD peptides were shown to induce endothelium-independent dilation rather than constriction in rat cremaster skeletal first-order arterioles (17). The dilation was associated with a decrease of smooth muscle [Ca^{2+}], and rhythmic calcium spikes (7). The renal vasculature is well known for its uniqueness in its response to vasoactive agents. It is not a surprise to find renal vasculature responding to the same agent in an opposite direction compared with other vascular beds. Another example is adenosine, which causes constriction in renal arterioles but dilatation in other vascular beds (31). The finding of RGD-induced vasoconstriction in AA might have profound effects in the potential use of RGD peptides in ameliorating the tubular obstruction and leukocyte infiltration in acute renal failure, which counts on exogenous RGD peptides given intravenously to occupy the exposed integrins on the luminal surface of renal epithelial cells (10, 19). The RGD-induced constriction in AA might cause hypoperfusion and reduction of glomerular filtration rate while attempting to rescue tubular function. Whether RGD-induced constriction will increase the AA resistance in vivo will depend on the rate of NO released from the renal endothelium. Interestingly RGD binding sites were also demonstrated in renal endothelium during ischemic acute renal failure, which suggested abnormality of integrin receptors in vascular endothelial cells in acute renal failure (23).

In summary, in this study we used indirect immunofluorescence microscopy to demonstrate the presence of a variety of integrin subunits in the AA of rat kidney. The exogenous RGD-containing peptide induced a dose-dependent constriction in the isolated AA. This induced constriction was specific to the RGD motif, and the constriction was associated with an increase in the smooth muscle [Ca^{2+}].

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