PAR-2 elicits afferent arteriolar vasodilation by NO-dependent and NO-independent actions

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Trottier, Greg, Morley Hollenberg, Xuemei Wang, Yu Gui, Kathy Loutzenhiser, and Rodger Loutzenhiser. PAR-2 elicits afferent arteriolar vasodilation by NO-dependent and NO-independent actions. Am J Physiol Renal Physiol 282: F891–F897, 2002. First published December 4, 2001; 10.1152/ajprenal.00233.2001.—Proteinase-activated receptors (PARs) are a novel class of G protein-coupled receptors that respond to signals through endogenous proteinases. PAR activation involves enzymatic cleavage of the extracellular NH2-terminal domain and unmasking of a new NH2 terminus, which serves as an anchored ligand to activate the receptor. At least four PAR subtypes have been identified. In the present study, we used the in vitro perfused hydronephrotic rat kidney to examine the effects of activating PAR-2 on the afferent arteriole. The synthetic peptide SLIGRL-NH2, which corresponds to the exposed ligand sequence SLIGRL-NH2, which corresponds to the exposed ligand sequence SLIGRL-NH2, inactive at PAR-2) had no effect. This vasodilation was characterized by an initial transient component followed by a smaller sustained response. A similar pattern of vasodilation was seen when SLIGRL-NH2 was administered to isolated perfused normal rat kidney. The sustained component of the PAR-2-induced afferent arteriolar vasodilation was eliminated by nitric oxide (NO) synthase inhibition (100 μM nitro-L-arginine methyl ester). In contrast, the transient vasodilation persisted under these conditions. This transient response was not observed when afferent arterioles were preconstricted with elevated KCl, suggesting involvement of an endothelium-derived hyperpolarizing factor. Finally, RT-PCR revealed the presence of PAR-2 mRNA in isolated afferent arterioles. These findings indicate that PAR-2 is expressed in the afferent arteriole and that its activation elicits afferent arteriolar vasodilation by NO-dependent and NO-independent mechanisms.

Microcirculation; endothelium; proteinase; angiotensin II; potassium chloride; nitric oxide

Proteases such as trypsin and thrombin activate a novel class of G protein-linked receptors termed proteinase-activated receptors (PARs) (7, 21). Four members on this class of receptors, PAR-1, PAR-2, PAR-3, and PAR-4, have been described (15–17, 26, 34, 36). PARs are activated by enzymatic cleavage of the extracellular NH2 terminus to reveal a new NH2 terminus containing a specific amino acid sequence that acts as a tethered ligand for the receptor. PAR-2 is unique, in that it is activated by trypsin and mast cell-derived tryptase (8, 26) but not by thrombin (26). PAR-2 can also be activated by a synthetic peptide corresponding to the enzymatically exposed ligand sequence SLIGRL or SLIGRL-NH2 (26, 30–32) and is expressed in vascular and nonvascular smooth muscle (25, 31) and in vascular endothelium (22).

Previous investigations of renal PARs have focused primarily on the thrombin receptor PAR-1. Activation of PAR-1 was shown to stimulate mesangial cell proliferation (2) and is implicated in the inflammatory events of crescentic glomerular nephritis (9). Thrombin is likely involved in fibrin and extracellular matrix deposition during renal inflammation (11, 12), possibly by the activation of PAR-1. There is little information on the functional effects of renal PAR-2 activation. Nevertheless, PAR-2 is abundantly expressed in human (6) and mouse (26) kidney. Immunohistochemical studies demonstrate PAR-2 in renal epithelial cells, renal vascular endothelium, and vascular smooth muscle (4). PAR-2 has been shown to activate a Cl− conductance in cortical collecting duct cells (4). However, the effects of PAR-2 activation on the renal microcirculation have not been investigated. In the present study, using the peptide sequence specific for PAR-2, we determined the renal afferent arteriolar actions of PAR-2. The effects of PAR-2 activation under basal conditions and during angiotensin II- and KCl-induced vasoconstriction were investigated using the in vitro perfused hydronephrotic rat kidney. Our findings indicate that PAR-2 is a potent vasodilator of the afferent arteriole and that its actions involve NO-dependent and NO-independent mechanisms.

Methods

In vitro perfused hydronephrotic kidney studies. The in vitro perfused hydronephrotic rat kidney model (33) was

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employed to study the effects of PAR-2 activation on the renal afferent arteriole. Unilateral hydronephrosis was induced by ligating the left ureter of young male Sprague-Dawley rats under halothane-induced anesthesia. Kidneys were harvested after 6–8 wk, when the tubular atrophy allows direct visualization of the microvasculature. Animals were anesthetized with methoxyflurane, the left renal artery was cannulated in situ, and the kidney was excised with continuous perfusion.

Kidneys were perfused using a single-pass perfusion system (33). Perfusion pressure, monitored within the renal artery, was maintained at 80 mmHg by an automated pressure controller (Biomedical Instruments, University of California). The perfusate consisted of Dulbecco’s modified Eagle’s medium (Sigma) containing 30 mM bicarbonate, 5 mM glucose, and 5 mM HEPES (GIBCO). The medium was equilibrated with 95% air-5% CO2. Temperature was maintained at 37°C and pH at 7.4. Pharmacological agents were added directly to the perfusate.

**In vitro perfused normal kidney studies.** The isolated perfused normal rat kidney model was used to assess the effects of PAR-2 activation in the nonhydronephrotic rat kidney. For these studies, kidneys were harvested from 300-g male Sprague-Dawley rats under methoxyflurane-induced anesthesia. Kidneys were perfused with an identical perfusate, and the kidney was excised with continuous perfusion.

**RESULTS**

The peptide sequence SLIGRL-NH2 corresponds to the activation sequence within the extracellular NH2-terminal domain of the PAR-2 receptor and is a PAR-2-selective agonist. In the in vitro perfused hydronephrotic rat kidney model, SLIGRL-NH2 had no effect on basal afferent arteriolar diameter at concentrations as high as 30 μM: 16.9 ± 0.2 μm in control, 17.3 ± 0.3 μm after 10 μM SLIGRL-NH2 (P > 0.1, n = 4), and 17.4 ± 0.3 μm for 30 μM SLIGRL-NH2 (P > 0.5, n = 4). When administered after NO synthase inhibition (100 μM l-NAME), 10 μM SLIGRL-NH2 elicited a modest vasoconstriction, reducing afferent arteriolar diameter from 16.1 ± 0.5 to 14.2 ± 1.0 μm (P < 0.05, n = 8).

When administered during angiotensin II-induced vasoconstriction, SLIGRL-NH2 elicited a biphasic vasodilator response. As shown in Fig. 1, the response was characterized by an initial peak response, which was followed by a reduced, but sustained, vasodilation. In this experiment, angiotensin II reduced afferent arteriolar diameter from 16 to 8 μm. SLIGRL-NH2 (10 μM) elicited a modest vasodilator response, reducing afferent arteriolar diameter from 16.1 ± 0.5 to 14.2 ± 1.0 μm (P < 0.05, n = 8).

**Materials.** The synthetic PAR-2 peptides SLIGRL-NH2 and LSIGRL-NH2 (>95% pure by HPLC and mass spectral criteria) were prepared by Immunosystems at BioChem Therapeutic (Laval, PQ, Canada) or by the Peptide Synthesis Facility at the Faculty of Medicine, University of Calgary. Stock solutions were prepared in 25 mM HEPES, pH 7.4, and concentrations were verified by quantitative amino acid analysis.
μM) evoked a strong initial vasodilation, which peaked at 14 μm. However, the diameter spontaneously returned to 10 μm within 5 min. Concentration-response studies were conducted using the PAR-2-activating peptide. Because PAR-2 can exhibit desensitization (10), we could not rely on a cumulative dose-response approach. To circumvent this potential issue, we tested one concentration of the peptide, allowed the preparation to recover for ≥1 h, and then tested a second concentration. Thus only two concentrations were examined in each preparation. In this series, 0.1 nM angiotensin II reduced afferent arteriolar diameter from 16.7 ± 0.4 to 6.8 ± 0.5 μm. Figure 2 summarizes the mean concentration-response data obtained using this approach. At 3 μM, SLIGRL-NH2 resulted in an increase in afferent arteriolar diameter to a peak of 12.0 ± 1.2 μm (P < 0.05) followed by a sustained dilation to 10.9 ± 1.4 μm (P < 0.05, n = 6). At 30 μM, SLIGRL-NH2 increased afferent arteriolar diameter to a peak of 16.5 ± 0.5 μm, corresponding to a full reversal of the angiotensin II-induced vasoconstriction. The diameter subsequently returned to a sustained value of 13.1 ± 2.0 μm (P < 0.05 vs. angiotensin II alone, n = 4). The IC50 for the peak component of the vasodilation was ~3 μM. As a control, we examined the effects of LSIGRL-NH2, a peptide in which the first two amino acids are reversed. LSIGRL-NH2 has no activity at PAR-2. In these studies, 0.1 nM angiotensin II reduced afferent arteriolar diameter from 16.1 ± 0.4 to 7.3 ± 0.8 μm. LSIGRL-NH2 (10 μM) had no vasodilatory effect (7.4 ± 0.8 μm, P > 0.5 compared with angiotensin II alone, n = 4). After the administration of LSIGRL-NH2, which did not elicit a response, the application of 10 μM SLIGRL-NH2 evoked prompt vasodilation.

To determine whether the vasodilatory actions of SLIGRL-NH2 required NO synthesis, a separate series of kidneys were pretreated with 100 μM L-NAME. L-NAME treatment did not significantly alter basal diameter: 17.1 ± 0.7 and 16.7 ± 0.6 μm for control and L-NAME, respectively (P > 0.4, n = 4). As depicted in Fig. 3, in the presence of L-NAME, SLIGRL-NH2 evoked only the transient component of the vasodilation. In this setting, angiotensin II reduced diameter from 16.7 ± 0.6 to 8.2 ± 1.4 μm. The application of 10 μM SLIGRL-NH2 caused a transient increase in diameter to 13.3 ± 0.6 μm (P < 0.05 vs. angiotensin II alone). Diameter returned to 6.7 ± 1.7 μm (P > 0.05) within 5 min.

We observed similar effects of L-NAME on acetylcholine-induced afferent arteriolar vasodilation (13, 33, 35). The transient vasodilation that is evoked by acetylcholine in this setting has been suggested to be mediated by an endothelium-derived hyperpolarizing factor (EDHF) and is prevented by elevated extracel-
lular potassium concentration (13, 35). As shown in Fig. 4, elevated extracellular potassium concentration (25 mM) also abolished the l-NAME-insensitive component of the SLIGRL-NH₂-induced vasodilation. In controls (without l-NAME or ibuprofen), KCl reduced afferent diameter from 18.4 ± 1.3 to 5.3 ± 0.6 μm. The addition of 10 μM SLIGRL-NH₂ reversed this response by 64 ± 7%, increasing diameter to 13.6 ± 1.0 μm (n = 5, P < 0.01). In the presence of 10 μM ibuprofen, KCl reduced diameter from 16.0 ± 0.9 to 4.3 ± 0.5 μm and SLIGRL-NH₂ increased diameter to 12.6 ± 0.9 μm (n = 5, P < 0.05), corresponding to a 71 ± 5% dilation (P = 0.45 vs. control). In the presence of both l-NAME and ibuprofen, KCl reduced diameter from 16.8 ± 0.5 to 4.7 ± 0.1 μm. In this setting, SLIGRL-NH₂ was without effect: 4.8 ± 0.2 μm, a 0.6 ± 1.6% dilation (n = 5). These data are summarized in Fig. 4, C and D.

We next examined the responses of normal kidneys to SLIGRL-NH₂ perfused in vitro under identical conditions to determine whether PAR-2 activation elicited similar actions in the nonhydronephrotic kidney. As shown in Fig. 5, 10 μM SLIGRL-NH₂ also elicited a biphasic vasodilation in the normal kidney. In five such preparations, 0.1 nM angiotensin II reduced renal perfusate flow from 15.1 ± 1.9 to 6.3 ± 0.9 ml·min⁻¹·g⁻¹. The addition of 10 μM SLIGRL-NH₂ increased mean flow to a peak value of 11.8 ± 1.6 ml·min⁻¹·g⁻¹, which spontaneously abated to 10.0 ± 1.1 ml·min⁻¹·g⁻¹ within 10 min. These data, normalized to the percentage of basal perfusate flow, are presented in Fig. 5. We interpret these observations as indicating that the PAR-2-activating peptide also evoked vasodilation in the normal kidney and that the temporal character of this response is similar to that in the afferent arteriole of the hydronephrotic kidney preparation.

Finally, we investigated whether the afferent arteriole expresses message for PAR-2. We used RT-PCR to determine whether mRNA for PAR-2 is present in afferent arterioles that were isolated from normal rat kidneys (20). As depicted in Fig. 6, we were able to detect a 190-bp product from the cDNA isolated from the afferent arteriole using the PAR-2 primers. This product was sequenced and shown to correspond to the rat PAR-2 sequence (31).

**DISCUSSION**

The major finding of this study is that PAR-2 activation elicits afferent arteriolar vasodilation by NO-dependent and NO-independent mechanisms. Although PAR-2 is abundantly expressed in the kidney (6, 26), there is little current information on the impact of PAR-2 activation on renal function. Bertog et al. (4) demonstrated, using antibody probes to PAR-2, that the receptor is present on murine renal vascular and tubular tissues. These investigators found PAR-2 to be localized to the basolateral side of cortical collecting duct cells and found that PAR-2 activation stimulated an apical Cl⁻ current, suggesting a functional role in the distal portion of the nephron. Our findings indi-
cate that PAR-2 stimulation can also modulate renal function by evoking afferent arteriolar vasodilation through mechanisms that are classically associated with endothelial cell activation.

Previous studies using large arteries demonstrated endothelium-dependent vasodilatory responses to PAR-2-activating peptides (30, 31). The present report is the first to examine PAR-2 responses of the renal afferent arteriole. Our studies examining the flow response of the normal rat kidney and the afferent arteriolar response of the hydronephrotic kidney model demonstrate PAR-2-mediated renal vasodilation. The results of our RT-PCR assay confirm that the normal rat afferent arteriole expresses message for this receptor. In other vascular preparations where NO synthase was inhibited with L-NAME, the relaxation responses to PAR-2 activation were abolished (31). In the afferent arteriole, we found that NO synthase inhibition eliminated only the sustained component of the PAR-2 vasodilatory response. The initial peak afferent arteriolar vasodilation evoked by SLIGRL-NH2 was not affected by L-NAME but was abolished by 25 mM KCl. Elevated KCl eliminates vasodilator responses mediated by potassium channel activation (28). We suggest that the transient dilation elicited by PAR-2 activation may be mediated by an EDHF, whereas the sustained response required NO formation. EDHF responses to other endothelium-dependent vasodilators exhibit a similar transient character (3, 35). Although our studies cannot rule out a direct effect of PAR-2 on the afferent arteriolar smooth muscle, PAR-2-mediated vasodilation in other vascular preparations has been shown to be eliminated when the endothelium is mechanically removed (30, 31).

We previously showed that the afferent arteriolar response to acetylcholine is sustained for ≥10–15 min (33). By contrast, the afferent arteriolar vasodilation induced by PAR-2 activation was biphasic, in that it was composed of an initial transient component followed by a reduced sustained phase. Only the sustained component was blocked by L-NAME. The initial transient response to 30 μM SLIGRL-NH2 attained a near-maximal afferent arteriolar vasodilation, whereas the sustained component was less robust (Figs. 1 and 2). A similar profile in the perfusate flow response was observed when SLIGRL-NH2 was administered to the in vitro perfused normal kidney (Fig. 5). By contrast, SLIGRL-NH2 elicited a monophasic dilation of conduit arteries (1, 30). One possible explanation for this temporal profile is that, initially, a transient EDHF-dependent component of the PAR-2 vasodilatory response predominates over the sustained NO-dependent component. Alternatively, PAR-2 desensitization could contribute to a diminishing effectiveness of SLIGRL-NH2 during exposure to the agonist. Receptor desensitization has been associated with enzymatic and agonist peptide activation of PAR-2 (5, 10) but was not observed in PAR-2-mediated relaxation of endothelium-intact rat aorta (1). Moreover, in renal M-1 cells, only enzymatic activation induces desensitization, whereas agonist peptide activation of the receptor does
not (4). Finally, it is possible that SLIGRL-NH₂ may concurrently activate another receptor population that is associated with a delayed contractile effect. In support of the latter postulate, we observed an SLIGRL-NH₂-induced afferent arteriolar vasoconstriction after NO synthase inhibition. Previous studies using other vascular preparations have demonstrated that SLIGRL-NH₂ elicits endothelium-dependent (30, 32) and endothelium-independent (23) vasoconstriction.

We can only speculate on the physiological and pathophysiological implications of our findings. In vivo, PAR-2 may be enzymatically activated by serine proteases such as trypsin. However, PAR-2 has also been shown to be activated by mast cell-derived tryptase (8, 24), and endothelial PAR-2 can be elevated by inflammatory stimuli (27). Mast cells are present in very low numbers in the normal kidney, but the population of renal mast cells increases in conditions associated with chronic inflammation. Increases in renal mast cells have been demonstrated to be associated with glomerulonephritis, diabetic nephropathy, and renal graft rejection (14, 18, 29). During inflammatory events, proteinases are released and PAR-2 itself may be upregulated (27). PARs would likely be activated in these settings (8, 9). Lee et al. (19) reported that urine from patients with acute and chronic renal failure contains significant proteinase activity, whereas that from normal controls does not. Our findings suggest that proteinase activation of PAR-2 in the renal microcirculation would be associated with renal vascular NO production and a reduction in afferent arteriolar tone.

In summary, the present study demonstrates, for the first time, a functional response of the renal afferent arteriole to PAR-2 activation. Our findings suggest that PAR-2 elicits afferent arteriolar vasodilation by NO-dependent and NO-independent mechanisms. We suggest that the latter involves an EDHF-like response, in that it is prevented by elevated external potassium. These findings suggest that proteinase activation of renal microvascular PAR-2 would result in renal vasodilation. The physiological or pathophysiological role of this mechanism and the identification of renal proteases that might activate renal PAR-2 receptors in situ are important issues awaiting further study.

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REFERENCES


