Proteinase-Activated Receptors 1 and 2 Exert Opposite Effects on Renal Renin Release

Klaus Höcherl, Melanie Gerl, Frank Schweda

Abstract—Proteinase-activated receptors (PARs) 1 to 4 are highly expressed in the kidney and are involved in the regulation of renal hemodynamics and tubular function. Since intravascular infusion of the proteinase thrombin, which activates PARs, has been shown to decrease plasma renin activity in rats, we investigated the effects of the respective PAR subtypes on renin release using the isolated perfused mouse kidney model. Thrombin dose-dependently reduced perfusate flow and inhibited renin secretion rates (RSRs) that had been prestimulated by the β-adrenoreceptor agonist isoproterenol. The suppression of RSRs was prevented by the selective PAR1 inhibitor SCH79797, and direct activation of PAR1 by TFLLR mimicked the effects of thrombin on RSRs and vascular tone. Moreover, TFLLR suppressed the stimulations of RSRs in response to the loop diuretic bumetanide, to prostaglandin E2, or to a decrease in renal perfusion pressure but not in response to a reduction in extracellular calcium. The PAR2-activating peptide SLIGRNLH2 concentration dependently increased RSR and perfusate flow. The stimulation of RSRs by SLIGRL was markedly attenuated by N\textsuperscript{G}-nitro-L-arginine methyl ester, suggesting an NO-dependent mechanism. Activation of PAR4 by AYPGKF did not modulate RSRs or perfusate flow. PAR1 and PAR2 immunoreactivity were detected in the juxtaglomerular region and were colocalized with renin immunoreactivity. Our data provide evidence that PAR1 activation inhibits renal renin secretion and induces renal vasoconstriction, whereas PAR2 activation stimulates renin release and induces vasodilation mainly via the release of NO. (Hypertension. 2011;58:611-618.) ● Online Data Supplement

Key Words: renin • thrombin • juxtaglomerular apparatus • calcium • NO • angiotensin

Proteinase-activated receptors (PARs) belong to a family of G protein–coupled receptors that are proteolytically activated by a variety of proteinases, which cleave the extracellular N-terminus of PARs. The resulting new N-terminal domain of PARs acts as a tethered ligand binding to a site on the second extracellular loop of the receptor to initiate its autoactivation.\textsuperscript{1} Up to now, 4 subtypes of PARs have been described, PAR1, PAR2, PAR3, and PAR4. Thrombin activates PAR1, PAR3, and PAR4. PAR1 can also be activated by matrix metalloproteinase 1, plasmin, and activated protein C. PAR2 is activated by trypsin, trypstatine, and kallikreins, for example.\textsuperscript{2} Synthetic peptides, like TFLLR-NH\textsubscript{2} (PAR1), SLIGRL-NH\textsubscript{2} (PAR2), and AYPGKF-NH\textsubscript{2} (PAR4), corresponding with the tethered ligands of PARs, are commonly used to investigate specific receptor functions.\textsuperscript{1} PARs are thought to play critical roles in vascular homeostasis, coagulation, and inflammation.\textsuperscript{1,3} The PAR1 receptor, for example, mediates several cellular functions of thrombin, such as the induction of platelet aggregation, cell proliferation, and vascular contractility. The PAR2 receptor mediates proliferation and modulates vascular tone, whereas the functional roles of PAR3 and PAR4 appear to be mostly limited to platelets.\textsuperscript{1}

All 4 PARs are expressed within the kidney.\textsuperscript{4-9} Immunohistochemical studies revealed PAR1 and PAR2 expression in renal vascular and tubular cells.\textsuperscript{5,8,9} Activation of PAR1 induces renal vasoconstriction and reduces glomerular filtration rate, whereas activation of PAR2 causes vasodilation and increases glomerular filtration rate in isolated perfused rat kidneys preconstricted with angiotensin II.\textsuperscript{5} Furthermore, activation of basolateral PAR2 stimulates chloride secretion in renal cortical collecting duct cells.\textsuperscript{4}

The release of renin from the juxtaglomerular cells of the kidney is the rate-limiting step within the renin-angiotensin system, which plays a crucial role in the control of systemic blood pressure and in the control of salt and water balance. Renin secretion is regulated by a complex interplay of several systemic and local factors. Several renal vasoconstrictors, such as angiotensin II, endothelin, or vasopressin, suppress renin secretion, whereas vasodilators, such as prostacyclin, prostaglandin E2 (PGE\textsubscript{2}), or NO, stimulate it.\textsuperscript{10} As mentioned above, activation of PAR1 induces vasoconstriction, whereas activation of PAR2 has the opposite effect,\textsuperscript{5} leading to our hypothesis that PARs might also control renin release. This hypothesis is supported by the results of a previous study performed in rats in which intravascular infusion of thrombin reduced renal blood flow and plasma renin activity.\textsuperscript{11} Given the impact of thrombin on activation of PAR1, we speculated that PAR activation may modulate renin release. Therefore,
we investigated the effects of PAR activation on renin secretion and on perfusate flow in the isolated perfused mouse kidney model.

Materials and Methods

Experimental Animals

All of the animal experiments were performed according to the Guidelines for the Care and Uses of Laboratory Animals published by the National Institutes of Health and were approved by the local ethics committee.

Isolated Perfused Mouse Kidney and Immunohistochemistry

Kidneys of male C57BL/6 mice were perfused ex situ as described in detail previously.12 If not stated otherwise, perfusion was performed at a constant perfusion pressure (90 mm Hg). Samples of the venous perfusate were collected every 2 minutes for the determination of renin activity. Renin secretion rates (RSRs) were calculated as the product of the renin activity and the venous flow rate (milliliters per minute per gram of kidney weight). Immunohistochemistry was basically performed as described previously.13 Mouse kidneys were fixed in 4% paraformaldehyde solution by retrograde perfusion through the abdominal aorta. Immunolabeling was performed on 5-μm paraffin sections. Sections were incubated with a PAR1 antibody or anti-PAR2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and an antirenin antibody (Davids Immunotechnologie, Regensburg, Germany) in Tris-buffered saline containing 5% horse serum and 0.3% Triton-X100 overnight at 4°C, followed by incubation with combinations of cyanine 2 or tetramethylrhodamine B isothiocyanate secondary antibodies (Dianova, Hamburg, Germany) for 90 minutes at room temperature. For details please see the online Data Supplement at http://hyper.ahajournals.org.

Statistical Analyses

In the isolated perfused kidney experiments, the last 2 values obtained within an experimental period were averaged and used for statistical analysis. Values are given as mean±SEM. All of the data are expressed as per gram of kidney weight. Differences between groups were analyzed by repeated-measurements ANOVA followed

Figure 1. Effect of increasing doses of thrombin (0.01 to 3 U/L) on perfusate flow (A) and renin secretion rates (B) in the presence of isoproterenol (10 nmol/L). Effect of thrombin (3 U/L) on perfusate flow (C) and renin secretion rates (D) in the presence of the proteinase-activated receptor (PAR) 1 antagonist SCH79797 (10 μmol/L). Values are mean±SEM of 5 experiments. *P<0.05 vs isoproterenol.
by Bonferroni post hoc test using GraphPad Instat3 software. P<0.05 was considered statistically significant.

Results

Effect of Thrombin on Perfusate Flow and RSR

Because RSRs from isolated perfused mouse kidneys are rather low under baseline conditions, the β-adrenoreceptor agonist isoproterenol (10 nmol/L) was added to the perfusate, thereby mimicking sympathetic activation. Isoproterenol increased perfusate flow and RSR at a constant perfusion pressure (90 mm Hg), 1.3- and 6.4-fold, respectively (Figure 1A and 1B). Subsequently, thrombin was infused in increasing concentrations. At a concentration of 3 U/L, thrombin reduced perfusate flow and RSRs to 79% and 38% of isoproterenol levels, respectively (Figure 1A and 1B). Both effects were rapidly reversible after the infusion of thrombin was terminated. Because activation of PAR1 has been shown to induce renal vasoconstriction,3 we speculated that thrombin reduces renal perfusion and renin secretion via PAR1. Selective PAR1 blockade by SCH79797 (10 μmol/L) did not abrogate the vasoconstriction of thrombin (Figure 1C), whereas it abolished the inhibition of renin secretion in response to thrombin (Figure 1D).

Activation of PAR1 Decreases RSR and Perfusate Flow

Increasing doses of the PAR1-activating peptide TFLLR induced a significant reduction of perfusate flow and RSR to 40% and 26% of isoproterenol levels, respectively, at a concentration of 10 μmol/L (Figure 2). Both effects were completely reversible after the infusion of TFLLR was terminated.

To exclude that PAR1 activation only suppresses renin secretion that has been prestimulated by β-adrenoreceptors, RSRs were enhanced by other hormones and maneuvers such as PGE2, the loop diuretic bumetanide, or a decrease of perfusion pressure. PGE2 (10 μmol/L) increased perfusate flow 1.7-fold and stimulated RSR 11.1-fold. Activation of PAR1 by TFLLR in the presence of PGE2 induced vasoconstriction (Figure 3A) and inhibited RSR to 22% of PGE2 levels (Figure 3B). Similarly, the loop diuretic bumetanide (100 μmol/L) enhanced perfusate flow 1.7-fold and stimulated RSR 12.7-fold. Again, TFLLR reduced both renal perfusion and RSR (Figure 3C and 3D). The reduction of perfusion pressure from 90 to 50 mm Hg decreased perfusate flow to 50% of baseline levels and stimulated RSR 5.0-fold (Figure 3E and 3F). Again, TFLLR decreased perfusate flow and RSR to 50% and 19% of the levels for 50 mm Hg, respectively (Figure 3E and 3F). Finally, RSRs were stimulated by a low extracellular calcium concentration obtained by the addition of EGTA (3.1 mmol/L) to the perfusate. EGTA clearly increased perfusate flow and RSR (Figure 3G and 3H). However, the addition of TFLLR neither induced vasoconstriction nor inhibited RSR under the low extracellular calcium concentration (Figure 3G and 3H).

Activation of PAR2 Increases RSR and Perfusate Flow

The PAR2 activating peptide SLIGRL increased perfusate flow from isolated kidneys in a concentration-dependent manner, starting at a concentration of 0.1 μmol/L (1.5-fold of baseline) and reaching a plateau at 3 μmol/L (2-fold of baseline; Figure 4A). In parallel, SLIGRL stimulated RSR in a concentration-dependent manner, starting at a concentration of 0.1 μmol/L (2.6-fold of baseline) and reaching a plateau at 3 μmol/L (6.6-fold of baseline; Figure 4B).

The stimulation of RSR in response to SLIGRL was augmented in the presence of isoproterenol. Isoproterenol at a concentration of 3 mmol/L increased RSR 4.2-fold (Figure 4D). SLIGRL (10 μmol/L) elevated perfusate flow (1.5-fold of isoproterenol levels) and further increased RSR (12-fold of isoproterenol levels). In contrast, blockade of NO synthesis by Nω-nitro-L-arginine methyl ester (1 mmol/L) decreased the isoproterenol-induced perfusate flow and RSR to ∼50%. The addition of SLIGRL under blocked NO synthesis increased perfusate flow 1.4-fold but did not significantly increase RSR (Figure 4C and 4D).

Regulation of Renin Secretion by PAR Activation Is Not Dependent on Changes in Perfusate Flow

Because our data show that activation of PARs regulates both RSR and perfusate flow, it might be speculated that the
Figure 3. Effect of TFLLR (10 μmol/L) on perfusate flow (A, C, E, and G) and renin secretion rates (B, D, F, and H) in the presence of prostaglandin E₂ (PGE₂; 10 nmol/L; A and B), bumetanide (100 μmol/L; C and D), EGTA (3.1 mmol/L; G and H), or in response to a reduction in perfusion pressure (E and F). Values are mean±SEM of 4 to 6 experiments. *P<0.05 vs PGE₂, bumetanide, EGTA, or a reduction in perfusion pressure, respectively.
changes in renin release result from altered renal perfusion. However, perfusion of the kidneys at constant flow rates prevented neither the suppression of renin secretion by activation of PAR1 nor the stimulation of renin release by PAR2 activation, arguing against this possibility (Figure S1). Moreover, blockade of L-type calcium channels by amlodipine (5 μmol/L) resulted in a marked vasodilation and completely prevented a further increase in perfusate flow by the PAR2-activating peptide SLIGRL (Figure 5A). Despite of the blunted effects on renal hemodynamics, SLIGRL (10 μmol/L) markedly stimulated renin secretion in the presence of amlodipine (Figure 5B). Similarly, amlodipine significantly attenuated the vasoconstriction in response to PAR1 activation. TFLLR reduced perfusate flow by 45% in the absence and by 9% in the presence of amlodipine (P<0.05; Figure 5C). However, TFLLR suppressed RSR to similar extents in the absence and the presence of amlodipine, suggesting that its effects on renin release are not dependent on its hemodynamic effects (Figure 5D).

Effect of PAR4 Activation on RSR and Per fusate Flow

The PAR4-activating peptide AYPGKF did not alter basal perfusate flow and RSR or isoproterenol-induced perfusate flow or RSR (Figure S2).

Immunolocalization of Renin, PAR1, and PAR2

Renin immunoreactivity was found at the typical juxtaglomerular position. PAR1 immunoreactivity was found in association with JGE cells and appeared in a strong punctuated pattern. PAR2 immunoreactivity was associated with JGE cells, with preglomerular vessels, and with intraglomerular structures. Renin-producing cells exhibited a punctuated PAR2 immunoreactivity (Figure 6).

Discussion

Thrombin signaling through proteinase-activated receptors has a wide range of physiological and pathophysiologic
effects in the cardiovascular system, because it is involved in coagulation, inflammation, and vascular contractility.\textsuperscript{14} PAR1 and PAR2 contribute to the regulation of renal hemodynamics.\textsuperscript{5,15} It has been reported that activation of PAR1 induces renal vasoconstriction in isolated perfused rat kidneys under baseline conditions.\textsuperscript{5} These results are confirmed and extended by our data, because thrombin and the PAR1-activating peptide TFLLR also reduce renal perfusion that had been elevated by isoproterenol. In addition, TFLLR reverses the vasodilation induced by PGE\textsubscript{2} or by bumetanide, and it further decreases perfusate flow in response to a reduction in renal perfusion pressure. Within the vascular system, different effects for PAR1 activation have been reported. Activation of PAR1 can induce endothelium-dependent relaxation and direct contraction in coronary arteries.\textsuperscript{16,17} In addition, a direct contractile effect in vascular smooth muscle cells has been reported.\textsuperscript{18} Although these results imply that activation of PAR1 can result in either relaxation or constriction in vascular beds, our data, together with previous results obtained in isolated perfused kidneys of rats, indicate that PAR1 induces vasoconstriction in the renal vasculature.\textsuperscript{5} Because thrombin increases the intracellular free calcium concentration in endothelial and vascular smooth muscle cells,\textsuperscript{19,20} we investigated the relevance of calcium for the vasoconstriction induced by PAR1 activation. Reduction of extracellular calcium by the calcium chelator EGTA or blockade of potential operated calcium channels by amlo-dipine completely prevented or significantly attenuated the reduction of perfusate flow in response to TFLLR, suggesting that PAR1-dependent renal vasoconstriction may be calcium dependent.
The protease renin is the key enzyme of the renin-angiotensin system. The release of renin can be modulated by a variety of intrarenal and extrarenal factors. Our data show that PARs are involved in the regulation renin release, too. Thrombin markedly suppressed renin secretion from isolated perfused kidneys in a concentration-dependent and reversible fashion. The inhibition of renin secretion by thrombin is prevented by SCH79797, indicating a PAR1-dependent effect. In line with an inhibitory effect of PAR1 on renin secretion, the PAR1-activating peptide TFLLR markedly suppressed renin secretion that had been prestimulated by isoproterenol, PGE2, bumetanide, or by a reduction of perfusion pressure, all of them well-known regulators of renin release. Thus, our findings indicate that activation of PAR1 exerts an inhibitory effect on renin release. The release of renin from juxtaglomerular cells is controlled by 2 main intracellular signaling pathways. cAMP is the major stimulus for renin release, and, in contrast to most secretory cells, a rise in intracellular free calcium is associated with inhibition of renin release. In line with this so-called “calcium paradox” of renin secretion, renin release is increased by a calcium-free perfusate. In this situation of a low extracellular calcium concentration, TFLLR did not suppress RSR.

In contrast to activation of PAR1, the PAR2-activating peptide SLIGRL concentration-dependently increases perfusate flow, confirming previous observations, in which SLIGRL partially reversed the vasoconstriction induced by TFLLR or by angiotensin II. In line with those studies, blockade of NO synthesis by N-nitro-L-arginine methyl ester attenuated the increase in perfusate flow by SLIGRL. Because SLIGRL still induced a moderate vasodilation in the presence of N-nitro-L-arginine methyl ester, our findings support the concept of NO-dependent and -independent vasodilation by PAR2. In addition, activation of PAR2 by SLIGRL increases renin secretion in a dose-dependent manner. This stimulation is abolished in the presence of the NO inhibitor N-nitro-L-arginine methyl ester, which strongly suggests a central role for NO in the stimulation of renin secretion in response to PAR2 activation. NO is produced at 2 distinct sites in the immediate vicinity of renin-secreting cells, namely in endothelial cells of the afferent arteriole via endothelial NO synthase and in the cells of the macula densa of the distal tubule via neuronal NO synthase. NO is a positive stimulus for renin secretion, and both neuronal NO synthase- and endothelial NO synthase–derived NO have been shown to serve as tonic enhancers of renin release. Because PAR2 expression has been localized to afferent arterioles, and because PAR2 activation has been linked to the formation of NO, one may speculate that the activation of PAR2 increases the formation of NO in afferent arterioles, which, in turn, stimulates renin secretion from renin-secreting cells.

Our data show that activation of PAR1 reduced perfusate flow and suppressed renin secretion, whereas PAR2 activation resulted in an elevation of renal perfusion and a stimulation of renal renin release. However, several lines of evidence indicate that PAR activation can regulate renal renin secretion independent of its effects on renal perfusion. First, renin release is controlled by renal perfusion pressure rather than by blood flow, and kidneys are perfused at constant pressure in our experimental setup. Second, TFLLR suppressed and SLIGRL stimulated renin secretion even in kidneys perfused at constant flow. Third, blockade of L-type calcium channels by amlopidine significantly reduced the PAR-1–dependent increase in vascular resistance but did not attenuate PAR-1-dependent suppression of renin release. This result is in line with previous data indicating that L-type calcium channels are not involved in the suppression of renin secretion in response to angiotensin II. Moreover, amlopidine did not prevent the stimulation of renin release in response to SLIGRL, although its vasodilating effect was blunted.

Other than the influence of PAR1 and PAR2 activation on perfusate flow and RSR, we determined the effect of PAR4 activation. In line with previous observations, the activation of PAR4 did not modify perfusate flow. Moreover, PAR4 activation did not alter renin secretion.

**Perspectives**

Our data indicate that activation of PAR1 induces a marked renal vasoconstriction and suppression of RSRs and that activation of PAR2 increases perfusate flow and stimulates renin secretion. Although PAR1 and PAR2 are abundantly expressed in the kidney, we can only speculate on the (patho-)physiological role of these opposing effects. Dysregulation of the renin-angiotensin system, inflammation, and a prothrombotic tendency are frequently observed in chronic kidney disease. Therefore, one might assume a role for PARs in the dysregulation of the renin-angiotensin system in chronic kidney diseases. In vivo, PAR2 may be enzymatically activated by mast cell tryptase, for example. The number of mast cells, which is rather low under physiological conditions, increases in chronic inflammatory renal diseases, such as lupus nephritis, which is associated with an increase in renal renin expression. Whether PAR2 in fact plays a role in the activation of the renin system in inflammatory renal diseases will be investigated in future studies. Furthermore, because kallikreins have been reported to activate PAR2 in vitro, and because these proteases are expressed in the...
kidney, a role for these kallikreins in PAR2 activation might be assumed.31–34 Taken together, additional detailed research is required to shed light on important situations by which PARs are potentially involved in the regulation of renin release in vivo.

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Disclosures
None.

References
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Proteinase-Activated Receptors 1 and 2 Exert Opposite Effects on Renal Renin Release

Klaus Höcherl, Melanie Gerl, Frank Schweda

From the Institute of Physiology (K.H., M.G., F.S.), University of Regensburg, Regensburg, Germany

Running title: Renin and PARs

Correspondence to Dr. Klaus Höcherl, Institut für Physiologie, Universität Regensburg, Universitätsstr. 31, D-93040 Regensburg, Germany
Tel.: x49-(0)941-943-2940, Fax: x49-(0)941-943-4315
E-Mail: klaus.hoecherl@chemie.uni-regensburg.de
MATERIAL AND METHODS

Experimental Animals

All animal experiments were performed according to the guidelines for the care and use of laboratory animals published by the US National Institutes of Health and was approved by the local ethics committee. Male C57bl/6 mice, weighting 25-27g, were used for the experiments. Mice were obtained from Charles River (Sulzfeld, Germany).

Isolated perfused mouse kidney

The isolated perfused mouse kidney model has been described in detail previously. Briefly, the animals were anesthetized with an intraperitoneal injection of 12 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany) and 80 mg/kg ketamine-HCl (Curamed, Karlsruhe, Germany), the abdominal aorta was cannulated, the right kidney was excised, placed in a thermostated moistening chamber, and perfused at constant pressure (90 mmHg), if not stated otherwise. Using an electronic feedback control, perfusion pressure could be changed and held constant in a pressure range between 40 and 140 mmHg. In some experiments, kidneys were perfused at constant flow rates and changes in perfusion pressure were monitored. Finally, the renal vein was cannulated and the venous effluent was collected for determination of renin activity and venous blood flow. The basic perfusion medium consisted of a modified Krebs–Henseleit solution supplemented with 6 g/100 ml bovine serum albumin and with freshly washed human red blood cells (a 10% hematocrit). After constant perfusion pressure was established, perfusate flow rates usually stabilized within 15 min. Then, basal values were recorded for the indicated periods. Thereafter, stock solutions of the drugs to be tested were added as constant infusion to the perfusate at the indicated time point. For the determination of renin secretion rates, three samples of the venous effluent were taken in intervals of 2 min during each experimental period. Renin activity in the venous effluent was determined by radioimmunoassay (Byk & DiaSorin Diagnostics, Dietzenbach, Germany) as described previously. Renin secretion rates were calculated as the product of the renin activity and the venous flow rate (ml/min * g kidney weight).

Materials

SCH79797, TFLLR, SLIGRL and AYPGKF were purchased from Biotrend Chemikalien GmbH (Cologne, Germany). Thrombin and PGE$_2$ were purchased from Cayman Chemical (Ann Arbor, MI, USA). Isoproterenol, bumetanide, amlodipine, EGTA and L-NAME were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany).

Immunohistochemistry

Immunohistochemistry for renin, PAR1 and PAR2 was basically performed as described previously. Mouse kidneys were fixed in 4% paraformaldehyde solution by retrograde perfusion through the abdominal aorta. Immunolabeling was performed on 5-µm paraffin sections. In brief, sections were washed three times for 5 minutes in Tris-buffered saline (TBS). Next, slices were blocked in TBS containing 5% horse serum and 0.3% Triton-X100 for 2 hours at room temperature. Kidney sections were incubated with an PAR1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-PAR2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and an anti-Renin antibody (Davids Immunotechnologie, Regensburg, Germany) in TBS containing 5% horse serum and 0.3% Triton-X100 overnight at 4°C, followed by incubation with combinations of cyanine 2 or TRITC secondary antibodies (Dianova, Hamburg, Germany) for 90 min at room temperature, diluted 1:400 or 1:300, respectively. Slices were mounted with Dakocytomation Glycergel.
mounting medium (Dako, Hamburg, Germany) and viewed with an Axiovert Microscope (Zeiss, Jena, Germany). As a negative control, we used the secondary antibody without prior incubation with the primary antibody.

**Supplementary Results**

**Effect of PAR1 and PAR2 activation on perfusion pressure and RSR**

Isoproterenol (10 nmol/L) decreased perfusion pressure and increased renin secretion rate in kidneys perfused with a constant flow (Figures S1A and S1B). Activation of PAR1 by TFLLR increased perfusion pressure (1.6-fold of isoproterenol levels) and decreased RSR (20% of isoproterenol levels) (Figures S1A and S1B). Activation of PAR2 by SLIGRL decreased perfusion pressure (85% of isoproterenol levels) and increased RSR (8.6-fold of isoproterenol levels) (Figures S1C and S1D).

**Effect of PAR4 activation on RSR and perfusate flow**

Isoproterenol increased perfusate flow at a constant perfusion pressure (90 mmHg) 1.4-fold and renin secretion rate 22.6-fold (Figures S2A and S2B). Subsequently, the PAR4-activating peptide AYPGKF was infused in increasing concentrations. However, AYPGKF did not alter perfusate flow or RSR (Figures S2A and S2B).

**References**


Figure S1. Effect of TFLLR (A, B) and SLIGRL (C, D) on perfusion pressure (A, C) and renin secretion rates (B, D) from mouse kidneys perfused with a constant flow in the presence of isoproterenol (10 nmol/L). Values are means ± SEM of 5 experiments. ★ p<0.05 vs. isoproterenol.
Figure S2. Effect of AYPGKF (0.3 – 30 μmol/L) on perfusate flow (A) and renin secretion rates (B) from isolated, perfused mouse kidneys in the presence of isoproterenol (10 nmol/L). Values are means ± SEM of 5 experiments. *p<0.05 vs. isoproterenol.