ADP-ribosyl cyclase and ryanodine receptor activity contribute to basal renal vasomotor tone and agonist-induced renal vasoconstriction in vivo

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Thai TL, Fellner SK, Arendshorst WJ. ADP-ribosyl cyclase and ryanodine receptor activity contribute to basal renal vasomotor tone and agonist-induced renal vasoconstriction in vivo. Am J Physiol Renal Physiol 293: F1107–F1114, 2007. First published July 25, 2007; doi:10.1152/ajprenal.00483.2006.—An important role for the enzyme ADP-ribosyl cyclase (ADPR cyclase) and its downstream targets, the ryanodine receptors (RyRs), is emerging for a variety of vascular systems. We hypothesized that the ADPR cyclase/RyR pathway contributes to regulation of renal vasomotor tone in vivo. To test this, we continuously measured renal blood flow (RBF) in anesthetized Sprague-Dawley rats. Infusion of the ADPR cyclase inhibitor nicotinamide intrareurally at low doses inhibits angiotensin II (ANG II) and norepinephrine (NE)-induced vasoconstriction by 72 and 67%, respectively (P < 0.001). RBF studies in rats were extended to mice lacking the predominant form of ADPR cyclase (CD38). Acute renal vasconstrictor responses to ANG II and NE are impaired by 59 and 52%, respectively, in anesthetized CD38−/− mice compared with wild-type controls (P < 0.05). Intrarenal injection of the RyR activator FK506 decreases RBF by 22% (P > 0.03). Furthermore, RyR inhibition with ruthenium red attenuates ANG II and NE responses by 50 and 59%, respectively (P < 0.01). Given at higher doses, nicotinamide increases basal RBF by 22% (P > 0.001). Non-receptor-mediated renal vasoconstriction by L-type voltage-gated Ca2+ channels is also dependent on ADPR cyclase and RyRs. Nicotinamide and ruthenium red inhibit constriction by the L-type channel agonist BAY K 8644 by 59% (P > 0.02) and 63% (P > 0.001). We conclude that 1) ADPR cyclase activity contributes to regulation of renal vasomotor tone under resting conditions, 2) renal vasoconstriction induced by G protein-coupled receptor agonists ANG II and NE is mediated in part by ADPR cyclase and RyRs, and 3) ADPR cyclase and RyRs participate in L-type channel-mediated renal vasoconstriction in vivo.

vascular smooth muscle; intracellular calcium; L-type calcium channels; FK506; CD38

REGULATION OF RENAL HEMODYNAMICS is essential for the maintenance of fluid and electrolyte balance and arterial blood pressure. Recent rises in morbidity and mortality rates associated with obesity and hypertension have made apparent the urgency of understanding the molecular mechanisms of blood pressure regulation, resulting in discovery of complex, integrated Ca2+ signaling pathways for regulation of renal vascular resistance (RVR) (2, 48). Stimulation of these pathways leads to increases in cytosolic Ca2+ concentration ([Ca2+]) via activation of plasma membrane Ca2+ channels as well as Ca2+ release channels in the sarcoplasmic reticulum (SR). The latter include inositol triphosphate receptors (IP3Rs) and ryanodine receptors (RyRs). The wide variety of mechanisms involved in [Ca2+]i regulation make it possible for vascular smooth muscle cells (VSMCs) to generate individualized responses to different vasoconstrictor stimuli. For example, it has been suggested that >50% of angiotensin II (ANG II)-induced changes in [Ca2+]i in afferent arterioles occur by Ca2+ entry, whereas the actions of norepinephrine (NE) are more dependent on Ca2+ mobilization from internal stores (48).

The role of IP3Rs in agonist-induced regulation of vascular resistance has been extensively studied (22, 30, 47, 49). Considering less is known about the role of RyRs in agonist-induced constriction in renal and other vascular beds (8, 14). One means of RyR activation involves the enzyme ADP-ribosyl cyclase (ADPR cyclase) and generation of the second messengers cyclic ADP ribose (cADPR) and nicotinic acid ADP (NAADP) (19, 65). Evidence indicates ADPR cyclase is important in Ca2+ signaling in the renal vasculature. Cycle activity is high in lysates from VSMCs in renal microvessels and glomeruli (12, 33), and cADPR applied extracellularly to isolated permeabilized VSMCs from rat renal artery increases [Ca2+]i (33). Furthermore, cADPR contracts isolated rat interlobular arterioles (33), and inhibition of ADPR cyclase, cADPR, or RyRs attenuates Ca2+ responses to ANG II or endothelin-1 (ET-1) in isolated rat afferent arterioles (19, 20). These in vitro studies demonstrate a potential physiological role for the enzyme in the renal microcirculation.

Whereas in vitro stimulation of ADPR cyclase activity in VSMCs leads to activation of pathways implicated in vasoconstriction, the actions of this enzyme in endothelial cells are predicted to cause vasodilatation. Inhibition of ADPR cyclase, cADPR, or RyRs is reported to prevent production of nitric oxide, a potent vasodilator, in response to bradykinin or the Ca2+ ionophore A23187 in bovine coronary artery endothelium (65). In some cases, ADPR cyclase in VSMCs may lead to dilation rather than constriction. This is suggested by the facts that inhibition of cADPR in isolated rat renal arteries attenuates relaxation produced by urocortin (50).

The importance of the ADPR cyclase/RyR signaling pathway has not been determined in any vascular network in vivo. Although experiments in isolated cells and vessels provide useful information regarding the nature of ADPR cyclase, the seemingly opposite results of ADPR cyclase activation in VSMCs and endothelial cells in vitro do not integrate possible interactions between endothelial cells and VSMCs that occur in vivo. To address this deficiency, we tested the hypothesis that the ADPR cyclase pathway contributes to both basal renal vascular tone and vasoconstriction produced acutely by ANG II, norepinephrine (NE), and the L-type voltage-gated Ca2+...
channel agonist BAY K 8644. The effects of pharmacological inhibitors of ADPR cyclase and its downstream effectors, RyRs, were determined in acute renal blood flow (RBF) studies conducted on anesthetized rats. The effects of genetic deletion of ADPR cyclase were determined in acute RBF studies comparing mice lacking the predominant form of ADPR cyclase (51), CD38 (CD38 −/−), with wild-type animals.

**MATERIALS AND METHODS**

Sprague-Dawley rats were obtained from our local breeding facility. CD38 −/− mice on a C57BL6 background were obtained as breeding pairs from Dr. Fran Lund [Trudeau Institute, Saranac Lake, NY (45)] and bred locally. Wild-type mice of a similar background were obtained from the Jackson Laboratory as breeder pairs. All animals were cared for and used for research in accordance with institutional guidelines. Experiments were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill and performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the guidelines of the Animal Welfare Act. Animals were anesthetized with pentobarbital sodium (Nembutal, 50 – 60 mg/kg body wt ip for rats, 80–90 mg/kg body wt ip for mice; Abbott, Chicago, IL).

The surgical preparation for acute RBF studies in rats was performed as is standard for our laboratory (31). Briefly, the right femoral artery was catheterized for continuous measurement of mean arterial blood pressure (MAP). The right femoral vein was catheterized for infusion of bovine serum albumin (4.75% at 50 μl/min for a length of time equal to 25% of body weight, then reduced to 10 μl/min for the remainder of the experiment) and administration of subsequent doses of pentobarbital as needed. A tracheotomy was performed, and a curved catheter was inserted into the aorta and positioned at the opening of the left renal artery for direct intrarenal infusion of pharmacological agents. RBF was measured in the left renal artery by a flow probe (model 1RB; Transonic Systems, Ithaca, NY).

The surgical preparation for RBF studies in mice was previously established in our laboratory (7) and modified for the purposes of this study. Briefly, a pulled PE-10 catheter was inserted into the right femoral artery and attached to a pressure transducer (Statham P23 Db) for measurement of MAP. Two pulled PE-10 catheters were inserted into the femoral vein for bolus injections of ANG II and NE and continuous infusion of 2.4% bovine serum albumin (10 μl/min) throughout the experiment. A tracheotomy was performed using a PE-100 catheter. A flow probe (TS420, 0.5-V probe; Transonic Systems) was placed around the left renal artery for measurement of RBF. Animals were allowed to stabilize for 1 h before the start of an experiment. Hematocrit, urine flow, kidney weight, and body weight were measured to ensure consistency of animal conditions.

**Bolus injections.** For rats, bolus injections (10 μl) were given intrarenally in the manner standard in our laboratory (31). The following concentrations were used: ANG II, 0.38 μM (4 ng); NE, 9.75 μM (20 ng); FK506, 31 mM (250 μg); and BAY K 8644, 0.70 mM (2.5 μg). The same doses of ANG II and NE were used for 10-μl venous injection in mice.

**Pharmacological inhibitors.** We used nicotinamide to inhibit ADPR cyclase (9, 25, 34, 38). Nicotinamide is a by-product of the ADPR cyclase reaction(32) and shifts the reaction to produce NAD+ rather than cADPR or NAADP. Nicotinamide may also inhibit poly-(ADP-ribose) polymerase-1 (PARP-1) (42, 63). PARP-1 is found in both endothelial cells (58) and VSMCs (26) and is activated under situations of extreme stress (3). Since the animals used in our study were closely monitored for stable hemodynamic conditions, activation of PARP-1 is unlikely.

Ruthenium red was used to inhibit RyRs. The effects of ruthenium red on RyRs are well documented (13, 37). Ruthenium red may also inhibit Ca2⁺-activated K⁺ (BKCa) channels (64). Although these channels have been shown to be present in renal microvessels (15), BKCa channel inhibition would result in vessel contraction, and our studies show ruthenium red inhibits agonist-induced contraction, arguing that the predominant effect of ruthenium red is on RyRs.

Nicotinamide or ruthenium red was infused into the renal artery at 140 μl/min for 3 min before vasoconstrictor injection and continued for an additional 2 min. Multiple doses of these agonists were tested in the same animal. Concentrations of ruthenium red and nicotinamide were based on in vitro published concentrations (19, 21) and calculated as estimated final renal arterial concentration. Concentrations that altered MAP or RBF were not used to inhibit vasoconstrictive agents. The target plasma concentrations of nicotinamide and ruthenium red were 3 mM (6 mg·kg⁻¹·min⁻¹) and 5 μM (126 μg·kg⁻¹·min⁻¹) in the renal artery, respectively.

Inhibitors were given at the highest dose that did not significantly alter basal RBF or MAP. The estimated plasma concentration of nicotinamide (3 mM) is higher than the IC50 of nicotinamide on cyclase activities of sea urchin egg homogenates (1.5 mM) and ADPR cyclase isolated from Aplysia californica (0.04 mM) (53). Concentrations from 3 to 5 mM also have been shown to inhibit Ca2⁺ responses to β-NAD, the substrate for ADPR cyclase in sea urchin egg homogenates after 2 min (53), and to inhibit Ca2⁺ responses to ANG II in isolated afferent arterioles shortly after application (19). Infusion of nicotinamide at 6 mg·kg⁻¹·min⁻¹ is therefore likely sufficient to inhibit ADPR cyclase activity. Similarly, the estimated plasma concentration of ruthenium red (5 μM) is much higher than the IC50 published for ruthenium red on isolated RyR from rabbit skeletal muscle (29).

**Inhibition of basal vascular tone.** To evaluate activity of ADPR cyclase under resting conditions, a high dose of nicotinamide (12 mg·kg⁻¹·min⁻¹) was infused into the renal artery for 20 min at 140
μl/min. The animal was then allowed to rest for 20 min, during which time RBF returned to normal. Multiple doses were given to the same animal. Concentrations after which RBF did not return to normal or MAP changed were not used.

Pharmacological agents. Nicotinamide, ruthenium red, BAY K 8644, and ANG II were obtained from Sigma (St Louis, MO), FK506 was obtained from Cayman Chemical (Ann Arbor, MI), and NE was obtained from Abbott Laboratories (Chicago, IL). NE, ANG II, ruthenium red, and nicotinamide were dissolved in 0.9% NaCl. FK506, ANG II, and NE, bolus injections of multiple agents respectively. Because of the reversible nature of BAY K 8644, RBF, hematocrit, and urine flow were 112 ± 100.6 mmHg, 4.47 ± 0.23 ml/min·g kidney wt⁻¹, 43 ± 1%, and 32 ± 2 μl/min, respectively. Because of the reversible nature of BAY K 8644, FK506, ANG II, and NE, bolus injections of multiple agents could be given within the same animal. Only one inhibitor was used in an animal; each rat received either nicotinamide or ruthenium red.

ADPR cyclase activity mediates vasoconstriction produced by ANG II and NE. To determine the physiological importance of ADPR cyclase activity in agonist-induced renal vasoconstriction, we assessed the effect of the ADPR cyclase inhibitor nicotinamide on the acute renal response to ANG II. ANG II injected into the renal artery decreased RBF by 25 ± 3% in the control period (Fig. 1, A and B). Intrarenal infusion of nicotinamide (≤6 mg·kg⁻¹·min⁻¹) did not significantly alter baseline MAP or RBF but attenuated RBF responses to ANG II to a 7 ± 2% decrease (P < 0.001). This effect of nicotinamide was rapidly reversible; the RBF response to ANG II was completely restored after 10 min (Fig. 1B). These data demonstrate that ADPR cyclase activity strongly influences ANG II-mediated renal vasoconstriction in vivo.

We also tested nicotinamide’s ability to inhibit NE-induced renal vasoconstriction. NE injected into the renal artery produced an average 24 ± 3% decrease in RBF (Fig. 2, A and B). Short-term nicotinamide infusion inhibited the effect of NE by ~70%, as NE decreased RBF to 8 ± 1% of normal (P < 0.001). These data indicate that ADPR cyclase activation contributes to a significant percentage of NE-induced renal vasoconstriction. In the recovery period, the response to NE was greater than control.

ADPR cyclase activity contributes to renal vasoconstriction induced by L-type voltage-gated Ca²⁺ channels. Since nicotinamide similarly inhibited ANG II- and NE-induced contraction despite reported differences in the degree of Ca²⁺ mobilization (48), we tested whether basal activity of ADPR cyclase contributes to vasoconstriction produced independently of G protein-coupled receptors (GPCR). For this purpose, we evaluated renal vasoconstriction triggered by directly activating L-type voltage-gated Ca²⁺ channels using the agonist BAY K 8644. Intrarenal BAY K 8644 injection produced a 78 ± 7% decrease in RBF (Fig. 3, A and B). This response was inhibited by 59% with nicotinamide (P = 0.01), attenuating constriction to 33 ± 12% of baseline RBF. Again, the effects of nicotin-
RBF increased by 22% this dose, localized relaxation of the renal vasculature was observed (Fig. 4A). RBF increased by 22 ± 4% (P < 0.001) without changing MAP. Doses higher than 12 mg·kg⁻¹·min⁻¹ reduced MAP, indicating systemic effects, and therefore were not used. Our results demonstrate that nicotinamide inhibits the tonic renal actions of endogenous stimuli signaling through ADPR cyclase to maintain basal renal vascular resistance.

Stimulation of RyRs causes renal vasoconstriction. To determine whether RyRs function in the renal vasculature in vivo, we used FK506 to stimulate RyRs. FK506 activates RyRs in the same manner as cADPR, by binding and removing the inhibitory molecule FKBP12 or FKBP12.6 from the RyR (11). Injection of FK506 into the renal artery caused a 22 ± 6% constriction, compared with 5 ± 1% constriction due to vehicle alone (P = 0.02; Fig. 5). These results demonstrate the presence of functional RyRs, capable of contracting the renal vasculature upon activation.

RyRs mediate ANG II- and NE-induced renal vasconstriction. To further assess the physiological importance of RyRs in the renal vasculature, we determined the extent to which RyRs are involved in acute vasconstriction produced by ANG II and NE. Ruthenium red was used to inhibit RyRs. ANG II produced a 30 ± 5% decrease in RBF during control conditions (Fig. 6). Intrarenal infusion of ruthenium red did not alter basal RBF or MAP after 3 min but inhibited RBF responses to ANG II in a dose-dependent manner. The highest dose attenuated ANG II-mediated renal vasconstriction to a 15 ± 2% decrease in RBF (P < 0.01). The inhibitory effect of ruthenium red was reversible after 10 min.

Similarly, ruthenium red attenuated NE-induced renal vasconstriction. NE produced a 27 ± 2% decrease in RBF in the control period (Fig. 7). The highest dose of ruthenium red decreased the renal vascular response to 11 ± 2% (P < 0.01). Responses to NE returned to normal after 10 min. We conclude that RyRs contribute to GPCR-mediated renal vasconstriction in vivo.

RyRs mediate voltage-gated Ca²⁺ channel-induced renal vasconstriction. Because of the apparent similarity of RyR involvement in the vascular effects of ANG II and NE, we tested whether RyRs contribute to L-type voltage-gated Ca²⁺ channel-induced renal vasconstriction. Intrarenal injection of the L-type channel agonist BAY K 8644 produced a 78 ± 7% decrease in RBF (Fig. 8). This response was inhibited 63% by ruthenium red (P < 0.001), resulting in an attenuated RBF response of 29 ± 4%. BAY K 8644-induced RBF responses returned to normal after 10 min. These results demonstrate involvement of RyRs in renal vascular responses elicited by stimulating Ca²⁺ entry.

Genetic disruption of ADPR cyclase in mice leads to impaired renal vascular responses to ANG II and NE. To determine the effect of chronic inhibition of ADPR cyclase on ANG...
II- and NE-induced renal vasoconstriction, we compared RBF responses to ANG II and NE injected iv in wild-type and CD38−/− mice. CD38−/− mice showed impaired renal vascular reactivity to both ANG II and NE. Whereas ANG II and NE produced 30 ± 8 and 37 ± 6% decreases in RBF in wild type animals, mice lacking CD38 showed 8 ± 1 and 19 ± 4% decreases in RBF, respectively (Fig. 9, \( P < 0.05 \) for both). These data indicate that mutation of the ADPR cyclase CD38 results in attenuated renal vascular responses to ANG II and NE in vivo. Furthermore, our results demonstrate the requirement for ADPR cyclase in renal vasoconstriction is not specific to rats but exists in multiple species.

**DISCUSSION**

Our study is the first to provide information about the functional importance of the ADPR cyclase/RyR signaling pathway in the regulation of renal vascular resistance in vivo. Collectively, our results support the notion that ADPR cyclase and its intermediates are linked to renal vasoconstriction through activation of RyR and enhancement of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). Results obtained using pharmacological inhibitors and a knockout mouse model indicate that ADPR cyclase and RyRs mediate the renal vascular actions of ANG II and NE. In addition, the ADPR cyclase/RyR system contributes to renal vasoconstriction elicited by activation of Ca\(^{2+}\) entry through L-type channels independently of GPCR-associated second messengers, providing additional insight into the importance of this signaling system in Ca\(^{2+}\) metabolism and contraction of resistance arterioles in vivo. Our study reinforces our previous results showing that the ADPR cyclase/RyR pathway contributes significantly to the regulation of [Ca\(^{2+}\)]\(_i\) in individual afferent arterioles (18, 19) and extends these findings to the regulation of vascular resistance in the intact renal microcirculation during basal conditions as well as during GPCR agonist-induced renal vasoconstriction.

Basal levels of ADPR cyclase contribute to the maintenance of tonic renal vasomotor tone under resting conditions. The vasodepressor effects of nicotinamide have long been recognized (28, 60). More recent work shows that the mechanism is related to ADPR cyclase inhibition (5, 25). In the present study, infusion of high-dose nicotinamide into the renal artery produced a significant increase in RBF while MAP is stable, indicative of renal vasodilatation. The dilation observed is rapid in onset, increases over the observation period, and is readily reversible. One possible explanation for this biphasic response is that the large immediate increase in RBF reflects inhibition of ADPR cyclase and associated attenuation of downstream Ca\(^{2+}\) signaling. The secondary, slower progressive increase in RBF may represent a component of Ca\(^{2+}\) signaling affected by prolonged period of low [Ca\(^{2+}\)]\(_i\) caused by depletion of SR stores, associated with vasodilatation.

Basal activities of ADPR cyclase and RyRs are functionally important in the renal vasculature as evidenced by their contribution to the acute renal constrictor response to L-type Ca\(^{2+}\) channel activation. Nicotinamide and ruthenium red inhibited a significant portion of the renal vasoconstriction induced by the L-type Ca\(^{2+}\) channel agonist BAY K 8644, which was not dependent on GPCR activation of ADPR cyclase. It is not clear whether the BAY K 8644 response involves direct ADPR cyclase activation or the presence of basal tonic levels of RyRs.
cADPR and/or NAADP, which are sufficient to enhance RyR receptor activation by Ca\(^{2+}\) entry initiated by BAY K 8644. The latter seems more plausible; participation of ADPR cyclase activity in vasoconstriction induced by activation of L-type Ca\(^{2+}\) channels is most likely due to the impact of cADPR on CICR. Biochemical studies show that cADPR greatly sensitizes RyR to [Ca\(^{2+}\)], (40, 54) and acts predominantly, if not exclusively, by this mechanism (36) and that tonic ADPR cyclase activity is present in coronary artery homogenates (24, 25). Tonic activity of ADPR cyclase seems sufficient to provide cADPR to sensitize RyRs to respond to small changes in [Ca\(^{2+}\)], resulting in CICR and amplification leading to increased RVR. This idea is supported by recent data showing that specific inhibition of cADPR with 8-bromo-cADPR attenuates KCl-induced increases in [Ca\(^{2+}\)], in isolated afferent arterioles (17).

RyRs are generally considered downstream targets for the products of ADPR cyclase in other vascular beds (1, 21, 24). cADPR activates RyRs by removing FK506 binding proteins (FKBPs) that associate with the receptor (44, 61). When used clinically as an immunosuppressant, FK506 often produces a side effect of hypertension (46, 59). In this study, FK506 was continuously administered iv in the presence of nitro-L-arginine methyl ester for 1 h and resulted in a 47% decrease in RBF by 23% in an in situ autoperfused rat kidney(4) and to contract isolated rat and human renal arteries (52). To our knowledge, only one other study has investigated the effects of acute FK506 infusion on RBF in vivo (62). In this study, FK506 was continuously administered iv in the presence of nitro-L-arginine methyl ester for 1 h and resulted in a 47% decrease in RBF. Our study shows that effects of FK506 on RBF are localized to the kidney and occur when nitric oxide is present.

The actions of ADPR cyclase and RyRs are responsible for a majority of acute renal vasoconstriction elicited by ANG II and NE. We found that either nicotinamide or ruthenium red blocked up to 70% of agonist-induced renal vasoconstriction without changing baseline MAP. Furthermore, we showed that CD38\(^{-/-}\) mice have significantly attenuated renal vascular responses to ANG II and NE. It is unclear whether ADPR cyclase is directly activated by ANG II and NE, or whether basal levels of cADPR and/or NAADP enhance renal vascular responses to ANG II and NE. The previously reported literature has suggested that ADPR cyclase contributes to agonist-induced vasoconstriction. ADPR cyclase, cADPR, and RyRs participate in ANG II-mediated increases in [Ca\(^{2+}\)] in preglomerular resistance arterioles (19); those triggered by KCl-induced depolarization (17) and constrictor responses to NE are attenuated in aortic rings of CD38\(^{-/-}\) mice (39). RyRs also have been implicated in NE signaling in vascular myocytes (8). Our studies add to this pool of knowledge by demonstrating a functional role of ADPR cyclase and RyRs in the renal vasculature in vivo. Further studies are required to clarify the relative importance of agonist-induced activation of cADPR and/or NAADP, as well as the role of RyRs in the regulation of renal blood flow.
this signaling pathway compared with basal levels sufficient to accommodate CICR.

It is interesting to note that we observed a trend toward greater renal vascular responses to ANG II and NE during recovery after acute dose-dependent inhibition of the ADPR cyclase/RyR pathway. Sustained inhibition of Ca\(^{2+}\) mobilization may have resulted in accumulation of Ca\(^{2+}\) in SR stores that were unmasked as exaggerated Ca\(^{2+}\) release and agonist-induced contraction during the recovery period. This exaggerated response was particularly prominent in NE experiments involving nicotinamide.

Our results suggest that ADPR cyclase and RyRs may function importantly in both afferent and efferent arterioles. Activation of L-type Ca\(^{2+}\) channels is likely to increase renal vascular resistance by a primary action of Ca\(^{2+}\) entry in the preglomerular vasculature, predominantly afferent arterioles (10, 23). In contrast, efferent arterioles appear to have few, if any, L-type Ca\(^{2+}\) channels that are activated by BAY K 8644 or KCl-induced depolarization (10, 23, 35). Our results indicate that nicotinamide and ruthenium red inhibit >50% of the renal vascular response to BAY K 8644. In this regard, our results highlight the functional role of ADPR cyclase/RyR signaling in afferent arteriolar-mediated renal vasconstrictor initiated by Ca\(^{2+}\) entry.

ADPR cyclase may contribute to efferent arteriolar constriction as well. Earlier work on isolated rat afferent arterioles indicates an important role of the ADPR cyclase/RyR system in ANG II- and ET-1-induced increases in [Ca\(^{2+}\)] (16, 19). The importance of this system in Ca\(^{2+}\) signaling in the efferent arteriole is unknown. It is well accepted that the major resistance sites responsible for regulation of RBF are the small diameter afferent and efferent arterioles and that ANG II and NE constrict both sets of glomerular arterioles. The relative strength of contraction is reported to be equal (6, 55, 56) or with predominant effects on efferent arterioles (41). Since these studies suggest that at least 50% of renal vasconstriction takes place in the efferent arteriole, our findings that nicotinamide and ruthenium red inhibit 50–70% of ANG II- and NE-induced contraction raise the question that the ADPR cyclase/RyR signaling pathway may contribute to efferent arteriolar constriction as well.

In summary, we present RBF evidence that the ADPR cyclase/RyR pathway plays an important physiological role in the regulation of basal renal vascular resistance during resting conditions and in acute renal vasconstrictor responses elicited by ANG II, NE, and BAY K 8644 injection into the renal artery. This is the first study to document the functional importance of the ADPR cyclase/RyR pathway in the vasculature in vivo. Intrarenal infusion of high-dose nicotinamide to inhibit ADPR cyclase activity produces renal vasodilatation as evidenced by increased RBF and reduced RVR in the absence of a change in MAP. Lower doses of nicotinamide that did not affect basal RBF markedly attenuated the acute renal vasconstriction produced by intrarenal injection of ANG II, NE, or BAY K 8644. In all three cases, the constriction appeared to be mediated by RyR, since ruthenium red reduced the renal microcirculatory response to each agonist. Renal vascular reactivity to ANG II and NE was markedly attenuated in mice lacking the ADPR cyclase CD38, solidifying conclusions of vascular signaling based on nicotinamide inhibition of ADPR cyclase in rats. The BAY K 8644 studies provide insight into the functional importance of the ADPR cyclase/RyR pathway in Ca\(^{2+}\) signaling and CICR in renal vasconstriction that occurs independently of GPCRs.
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