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ADP-ribosyl cyclase and ryanodine receptors mediate endothelin ETA and ETB receptor-induced renal vasoconstriction in vivo

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Thai TL, Arendshorst WJ. ADP-ribosyl cyclase and ryanodine receptors mediate endothelin ETA and ETB receptor-induced renal vasoconstriction in vivo. Am J Physiol Renal Physiol 295: F360–F368, 2008. First published June 4, 2008; doi:10.1152/ajprenal.00512.2007.—ADP-ribosyl cyclase (ADPR cyclase) and ryanodine receptors (RyR) participate in calcium transduction in isolated afferent arterioles. We hypothesized that this signaling pathway is activated by ETA and ETB receptors in the renal vasculature to mediate vasoconstriction in vivo. To test this, we measured acute renal blood flow (RBF) responses to ET-1 in anesthetized rats and mice in the presence and absence of functional ADPR cyclase and/or RyR. Inhibitors of ADPR cyclase (nicotinamide) or RyR (ruthenium red) reduced RBF responses to ET-1 by 44% (P < 0.04 for both) in Sprague-Dawley rats. Mice lacking the predominant form of ADPR cyclase (CD38) had RBF responses to ET-1 that were 47% weaker than those seen in wild-type mice (P = 0.01). Selective ETA receptor stimulation (ET-1 + BQ788) produced decreases in RBF that were attenuated by 43 and 56% by nicotinamide or ruthenium red, respectively (P < 0.02 for both). ADPR cyclase or RyR inhibition also reduced vasoconstrictor effects of the ETB receptor agonist sarafotoxin 6c (S6c; 77 and 54%, respectively, P < 0.02 for both). ETB receptor stimulation by ET-1 + the ETA receptor antagonist BQ123 elicited responses that were attenuated by 59 and 60% by nicotinamide and ruthenium red, respectively (P < 0.01 for both). Nicotinamide attenuated RBF responses to S6c by 54% during inhibition of nitric oxide synthesis (P = 0.001). We conclude that in the renal microcirculation in vivo 1) ET-1-induced vasoconstriction is mediated by ADPR cyclase and RyR; 2) both ETA and ETB receptors activate this pathway; and 3) ADPR cyclase participates in ETB receptor signaling independently of NO.

endothelin-1; calcium signaling; afferent arteriole; kidney; CD38

ENDOTHELIN-1 (ET-1) is one of the most potent vasoconstrictors identified to date. Dysfunction in ET-1 regulation or receptor signaling has been implicated in several cardiovascular diseases, including atherosclerosis, coronary artery disease, congestive heart failure, cerebrovascular disease, and systemic and pulmonary hypertension and in acute and chronic renal disease (16, 29, 33, 40, 42, 43). ET-1 is thought to act primarily in a local paracrine fashion in the vasculature, being secreted from endothelial cells abluminally to act on nearby vascular smooth muscle cells (VSMC). Circulating levels of ET-1 appear to have a relatively minor influence on vascular tone. In this regard, it has been proposed that ETB receptors bind circulating ET-1 and provide a clearance function (43).

The renal vasculature is particularly responsive to ET-1 (30, 38). Acute intravenous administration of ET-1 decreases renal blood flow (RBF) in animals (23, 30) and increases renal vascular resistance in humans without affecting arterial pressure (39). The effects of ET-1 on RBF are due to contraction of preglomerular arteries and afferent and efferent arterioles as has been shown in specialized isolated vascular preparations (11, 25, 31) and in vivo (30, 38). Although ET-1 does not affect steady-state RBF autoregulation, ET-1 stimulation of NO production alters the dynamics of the preglomerular myogenic response (45).

ET-1 signals via two G protein-coupled receptors (GPCR): ETA and ETB (33, 37, 43). Stimulation of ETA or ETB receptors results in elevation of cytosolic calcium concentration ([Ca2+]i). ET-1 stimulates the production of inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 can bind to receptors (IP3Rs) on the sarcoplasmic reticulum (SR) to release Ca2+ while DAG activates PKC, resulting in downstream activation of myosin light chain kinase and cell contraction.

Most VSMC have SR with Ca2+-sensitive ryanodine receptors (RyR) that participate in Ca2+ mobilization. RyR can be activated by second messengers produced by the enzyme ADP ribosyl cyclase (ADPR cyclase) (1, 52). We have previously observed that this ADPR cyclase/RyR signaling pathway plays a significant role in mediating responses to ANG II in Ca2+ signaling in isolated afferent arterioles (13) and renal vasoconstriction in vivo (47). However, little is known about which GPCRs activate this particular second messenger system in specific vascular beds. Participation may depend on vessel size and function and the vascular bed. For example, it appears that thromboxane A2 (TXA2) signaling through the TP receptor does not involve ADPR cyclase and RyR in isolated renal arteries (46), whereas ADPR cyclase inhibition dilates TXA2-preconstricted coronary arteries (18). Interestingly, isolated aortas from mice genetically lacking the predominant form of ADPR cyclase (CD38) show normal contractile responses to ET-1, but contraction to the α-adrenergic receptor agonist phenylephrine is weaker than that seen in wild-type mice (36).
We find that α-adrenergic receptor-induced renal vasconstriction is dependent on ADPR cyclase and RyR in vivo (47).

Evidence indicates that ET-1 activates ADPR cyclase in VSMC of coronary, renal, mesenteric, and pulmonary beds. The ADPR cyclase inhibitor nicotinamide attenuates contraction of mesenteric arteries elicited by ET-1 (20). Evidence implicates both second messengers produced by ADPR cyclase in VSMC responses to ET-1. Incubation of coronary arterial myocytes with ET-1 stimulates production of the ADPR cyclase metabolite nicotinic acid ADP (NAADP) (53). In pulmonary arterial VSMC, Ca\(^{2+}\) responses to ET-1 are attenuated by bafilomycin A1, an inhibitor of NAADP-induced Ca\(^{2+}\) release (28). Selective inhibition of cADP ribose, a second metabolite formed by ADPR cyclase with 8Br-cADPR attenuates Ca\(^{2+}\) responses to ET-1 in rat renal afferent arterioles (12).

To our knowledge, the importance of ADPR cyclase/RyR signaling in mediating ET-1-induced vasconstriction has not been studied in vivo in any vascular bed. Due to the emerging importance of ADPR cyclase in GPCR signaling, we hypothesized that ADPR cyclase and RyR contribute to renal vascular responses to ET-1 in vivo. We determined the importance of this signaling pathway in responses to ET\(_A\) and ET\(_B\) receptor stimulation in the renal microcirculation.

**MATERIALS AND METHODS**

Sprague-Dawley rats, wild-type C57BL6 mice, and CD38\(^{-/-}\) mice on a C57BL6 background were obtained from our Chapel Hill breeding facility. CD38\(^{-/-}\) mice and wild-type control breeder pairs were originally obtained from Dr. Frances Lund (Trudeau Institute, Saranac Lake, NY) and Jackson Labs (Bar Harbor, ME), respectively. All animals were cared for and used in accordance with institutional guidelines. Protocols were approved by the local Institutional Animal Care and Use Committee. Animals were anesthetized using pentobarbital sodium (Nembutal; 50–60 mg/kg body wt ip for rats, 80–90 mg/kg body wt ip for mice, Abbott, Chicago, IL).

**Surgical procedures in rats.** The surgical preparation for measurement of RBF in rats was performed as is standard in our laboratory (26, 27, 47). The right femoral artery was catheterized using a PE-50 catheter for continuous measurement of mean arterial pressure (MAP) via a pressure transducer (Statham P23 dB). The right femoral vein was catheterized using three PE-10 catheters for infusion of bovine serum albumin (4.75% at 50 \(\mu\)l/min for min = body wt (g)/4, then reduced to 10 \(\mu\)l/min for the remainder of the experiment). Maintenance of anesthesia using pentobarbital sodium was required, and injection of \(N^o\)-nitro-L-arginine methyl ester (l-NNAME) when applicable. The bladder was catheterized, and a tracheotomy was performed. A curved PE-50 catheter was inserted into the left common iliac, passed up the aorta, and positioned such that the tip of the catheter was facing but not obstructing the left renal artery for administration of pharmacological agents. An ultrasonic flow transducer was placed around the left renal artery to measure RBF (model 1RB, Transonic, Ithaca, NY).

**Surgical procedures in mice.** Procedures for measuring RBF in mice were modified from those previously developed in our laboratory (5). A pulled PE-50 catheter was inserted into the right femoral artery for continuous measurement of MAP via a pressure transducer (Statham P23 dB). Pulled PE-10 catheters were inserted into a femoral vein for intravenous administration of bovine serum albumin (2.4%, 10 \(\mu\)l/min for the duration of the experiment) and ET-1. A tracheotomy was performed, and the bladder was catheterized. The left renal artery was freed from the renal vein, and RBF was measured by an ultrasonic flow transducer (0.5 V, Transonic).

**Assessment of renal vascular reactivity.** In rats, 10-\(\mu\)l bolus injections of ET-1 (7.5 ng) and sarafotoxin 6c (S6c; 7.5 ng) were given directly into the renal artery in the manner previously described (26, 27). Previous studies show that RBF and MAP responses to ET-1 and S6c recover, albeit slowly, over 30 min (26). As a result, multiple doses of ET-1 and/or S6c were given to the same animal. In mice, a 10-\(\mu\)l bolus of ET-1 (7.5 ng) was injected into a femoral vein. l-N-NAME (25 mg/kg in 1 ml/kg 0.9% NaCl) was injected into a femoral vein of rats 30 min before the start of an experiment.

Ruthenium red (126 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\)), an inhibitor of RyR, nicotinamide (6 mg·kg\(^{-1}\)·min\(^{-1}\)), an ADPR cyclase inhibitor, BQ123 (18.64 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\)), an ET\(_A\) receptor antagonist, and BQ788 (18.64 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\)), an ET\(_B\) receptor antagonist, were infused into the renal artery 3 min before and 5 min following ET-1 or S6c injection. These doses of nicotinamide and ruthenium red were based on an earlier study in which we established effective inhibition of ANG II- and NE-induced renal vasconstriction while neither agent affected basal RBF or MAP (47). It is important to note that complete inhibition was not reached at the doses used in the present study as higher amounts are known to produce more pronounced inhibition of ADPR cyclase, as evidenced by frank renal vasodilatation (47). Following the inhibitory period, the animal was allowed to recover for 10 min before a final assessment of reactivity. In all cases, the response during the recovery period returned to the control level, indicating rapid reversibility of inhibitors and stability of the preparation. The doses of ET receptor antagonists are known to effectively antagonize ET\(_A\) and ET\(_B\) receptors selectively (26). To avoid possible overlapping effects, each rat received only one inhibitor (nicotinamide or ruthenium red).

**Pharmacological agents.** ET-1, l-NNAME, nicotinamide, and ruthenium red were purchased from Sigma-Aldrich (St. Louis, MO). S6c, BQ123, and BQ788 were obtained from American Peptide (Sunnyvale, CA). All pharmacological agents were dissolved in 0.9% NaCl.

**Data analysis.** Data were collected using Labtech Notebook software, and graphs were created using SigmaPlot software. Statistical analyses of differences in RBF responses between control and experimental periods were performed by a paired Student’s \(t\)-test using SigmaStat software.

**RESULTS**

Results are reported for a total of 58 male Sprague-Dawley rats, averaging 7.3 ± 0.1 wk of age. In rats not treated with l-NNAME, RBF and MAP were 3.8 ± 0.2 ml·min\(^{-1}\)·g kidney wt\(^{-1}\) and 100 ± 4 mmHg, respectively. l-NNAME-treated animals showed an increased MAP (145 ± 3 mmHg, \(P < 0.001\)) and decreased RBF (3.0 ± 0.3 ml·min\(^{-1}\)·g kidney wt\(^{-1}\), \(P < 0.05\)).

**ET-1-induced renal vasoconstriction is dependent on ADPR cyclase activation and RyR.** To determine whether ADPR cyclase mediates renal vascular responses to ET-1, we gave intrarenal bolus injections of ET-1 to rats before and during intrarenal infusion of the ADPR cyclase inhibitor nicotinamide. ET-1 injection into the renal artery decreased RBF by 31 ± 3% (Fig. 1). This response was impaired in the presence of nicotinamide such that only a 17 ± 3% decrease in RBF was produced by the same amount of ET-1, a response that was decreased 45% from that observed in the control period (\(P < 0.01\)).

To test the importance of RyR, we compared renal vascular responses to ET-1 in the presence or absence of the RyR inhibitor ruthenium red in other animals. The 29 ± 4% decrease in RBF induced by ET-1 under control conditions was attenuated by ruthenium red. ET-1 produced a 16 ± 3% decrease in RBF in the experimental period (Fig. 2). Thus both ADPR cyclase and RyR appear to play a role in acute ET-1 responses in the renal microcirculation of normotensive rats.
Renal vascular responses to ETA receptor stimulation are mediated by ADPR cyclase and RyR. ETA and ETB receptors are both present in the renal microvasculature and mediate total RBF responses to ET-1 (7, 19, 26). As a result, we asked whether the importance of ADPR cyclase in the renal hemodynamic actions of ET-1 is selectively dependent on one ET receptor subtype over the other.

To specifically stimulate ETA receptors, we injected ET-1 into the renal artery of rats in the presence of the selective ETB receptor antagonist BQ788. In the absence of nicotinamide, the combination of ET-1 + BQ123 decreased RBF by 50 ± 5% (Fig. 3). When nicotinamide was infused into the renal artery to inhibit ADPR cyclase, the constrictor response to ETA receptor stimulation was markedly attenuated. During ADPR cyclase inhibition, ET-1 + BQ123 decreased RBF by 28 ± 3%, an attenuated response compared with ET-1 + BQ123 given without nicotinamide ($P < 0.01$). Similarly, RyR inhibition with ruthenium red attenuated the acute RBF response to ET-1 + BQ788 from 56 ± 10 to 25 ± 4% ($P < 0.02$, Fig. 4). Together, these data demonstrate a significant role for ADPR cyclase and RyR in ETA receptor-mediated renal vasoconstriction.

Vasoconstrictor responses to ETB receptor stimulation are dependent on activation of ADPR cyclase and RyR. To test the importance of ADPR cyclase and RyR in ETB receptor signaling, we injected the specific ETB receptor agonist sarafotoxin S6c into the renal artery. In other animals, a combination of the ETA antagonist BQ123 and ET-1 was tested. When given into the renal artery, S6c and ET-1 + BQ123 reduced RBF by 22 ± 4 (Fig. 5) and 13 ± 2% (Fig. 6), respectively. Thus, as has been previously demonstrated (26), we found that ETB receptor stimulation elicits net renal vasoconstriction. Nicotinamide markedly inhibited the vascular effects of both S6c and ET-1 + BQ123 such that only a 5 ± 2% decrease in RBF was observed in both groups ($P < 0.01$ for both). These findings suggest that ADPR cyclase participates in acute renal vasoconstrictor responses to selective ETB receptor stimulation in a healthy rat kidney.

Other experiments indicate that RyR participate in ETB receptor responses in the renal vasculature in vivo. Before administration of ruthenium red, S6c and ET-1 + BQ123 decreased RBF by 16 ± 3 (Fig. 7) and 9 ± 1% (Fig. 8), respectively. In the same animals, ruthenium red attenuated RBF changes during ETB receptor stimulation. During RyR inhibition with ruthenium red, S6c and ET-1 + BQ123 decreased RBF by 28 ± 3% (Fig. 9) and 19 ± 2% (Fig. 10), respectively. Together, these data demonstrate a significant role for RyR in ETB receptor-mediated renal vasoconstriction.
RBF responses to ET-1 are impaired in mice with decreased ADPR cyclase activity. The importance of ADPR cyclase in renal vascular responses to ET-1 was assessed in mice lacking the predominant form of ADPR cyclase (CD38). ET-1 injected intravenously produced a 19 ± 3% decrease in RBF in wild-type mice compared with a 10 ± 1% decrease in CD38−/− mice (P < 0.02, Fig. 10). This finding in mice reinforces our pharmacological studies in rats demonstrating the importance of ADPR cyclase in renal vascular responses to ET-1 in the rat.

DISCUSSION

Our study is the first to demonstrate a dependence of ET-1 signaling on ADPR cyclase activation and RyR in the microcirculation of any organ in rats and mice in vivo. Acute ET-1-induced vasoconstriction in the kidney is critically dependent on the activity of ADPR cyclase and RyR. This conclusion is based on results obtained using pharmacological inhibitors and a knockout mouse. We find that both ETA and ETB receptors utilize the ADPR cyclase/RyR signaling pathway to produce vasoconstriction of renal resistance arterioles in the rat.

ADPR cyclase activation by ETB receptors is independent of nitric oxide. In addition to vasoconstrictor properties of ETB receptors on VSMC, ETB receptors are present on endothelial cells of renal vessels (24, 48). When stimulated, these receptors produce nitric oxide (NO) and other dilator agents that buffer ET-1-induced renal vasoconstriction (22, 27). Since interactions between NO and ADPR cyclase have been reported in both VSMC and endothelial cells of nonrenal arteries (50, 54), we asked whether the effects of nicotinamide on ETB receptor-induced renal vasoconstriction persist in the absence of NO production. To test this, we evaluated the effect of ADPR cyclase inhibition on S6c responses during inhibition of NO synthase (NOS) using L-NAME. During L-NAME infusion, S6c decreased RBF by 28 ± 3% (Fig. 9). Nicotinamide attenuated the S6c response such that a 13 ± 2% decrease in RBF was observed in the experimental period (P < 0.005). These results suggest that ADPR cyclase activation by ETB receptor stimulation occurs in the absence as well as the presence of NO.
in rats. Experiments in mice demonstrate the importance of one ADPR cyclase family member, CD38 in particular, in ET-1-induced renal vasoconstriction. Our study extends previous work establishing the importance of ADPR cyclase and RyR in mediating \([Ca^{2+}]_i\) responses to ET-1 receptor stimulation in isolated renal afferent arterioles (12) and provides new information regarding the importance of this pathway in an integrated, natural environment.

We found that ADPR cyclase and RyR mediate a significant portion of acute renal vasoconstriction produced by ET-1. In our rat studies, pharmacological agents used to inhibit both ADPR cyclase and RyR attenuated renal vasoconstrictor responses to ET-1 by \(-45\%\). Nicotinamide and ruthenium red are widely used to inhibit ADPR cyclase and RyR, respectively. Nicotinamide is a byproduct of ADPR cyclase conversion of NAD\(^+\) to cADPR and NADP\(^+\) to NAADP (1, 32), and nicotinamide shifts the ADPR cyclase reactions to produce relatively small amounts of NAD\(^+\) and NADP\(^+\) rather than adding significantly to the pool of cADPR and NAADP. Although nicotinamide may also inhibit poly (ADP-ribose) polymerase-1 (PARP-1) (49), this is unlikely to be a major factor in our studies since PARP-1 is generally activated only under incidences of extreme stress (2) and our animals were closely monitored to ensure the most physiological conditions possible. As a commonly used inhibitor of RyR, ruthenium red binds directly to RyR, causing a conformational change that renders the channel inactive (34). Ruthenium red may also inhibit Cu\(^2+\)-activated K\(^+\) channels (BK<sub>Ca</sub>) (6, 34), known to be present in renal microvessels (35). If this were the primary action, however, ruthenium red would be predicted to enhance vasoconstriction rather than oppose it as is seen in our study.

Our results in rats were reinforced by studies in mice lacking the predominant form of ADPR cyclase (CD38\(^{-/-}\)). Three forms of ADPR cyclase are known to exist: the nonmammalian ADPR cyclase of the sea hare <i>Aplysia californica</i> and the two mammalian ADPR cyclases lymphocyte antigen CD38 and bone marrow stromal cell surface antigen BST-1 (CD157) (44). Whereas CD157 is present primarily on immune cell types and is upregulated during periods of stress, CD38 is more constitutively expressed and is expressed in several tissues (15). Isolated aortas without intact endothelium from CD38\(^{-/-}\) mice have impaired responses to phenylephrine, suggesting a vasoconstrictor role of CD38 linked to \(\alpha\)-adrenoco-
It is noteworthy that another form of ADPR cyclase sensitive to retinoic acid may exist in VSMC, although this form is poorly characterized (8). In an earlier study, we reported weaker than normal acute renal vascular responses to ANG II and NE in CD38−/− mice, suggesting that CD38 exists in resistance arterioles and mediates vasoconstriction in the kidney triggered by AT1 and adrenoceptors (47). Presently, we found that mice deficient in CD38 show attenuated renal vascular responses to intravenous injection of ET-1, supporting the notion that ADPR cyclase mediates acute ET-1-induced renal vasoconstriction in the mouse.

ET-1 responses were attenuated by 45% in the presence of either nicotinamide or ruthenium red. Similarly, responses to ET-1 in CD38−/− mice were 47% less than in wild-type controls. Collectively, these results indicate that the ADPR cyclase/RyR signaling pathway accounts for roughly half of the acute vasoconstriction of resistance arterioles in the kidney in response to ET-1. Previous in vitro results indicate that IP3 and its receptor play an important role in mediating [Ca2+]i responses to ET-1 in renal microvessels (3, 21). Although one cannot discern from the current data whether the contribution of the ADPR cyclase and IP3 pathways to ET-1-induced renal vasoconstriction works independently, it is probable that a synergistic effect exists between these two signaling mechanisms. RyR are Ca2+-sensitive receptors that can amplify [Ca2+]i from any source. Ca2+ release from IP3Rs on the SR may activate nearby RyR by Ca2+-induced Ca2+ release (CICR). cADPR may also contribute to the amplification of IP3R signaling since cADPR sensitizes RyR to CICR. In addition to cADPR, ET-1 stimulation of ADPR cyclase may produce NAADP in VSMC. Ca2+ exiting from lysosomal stores via stimulation of NAADP receptors may also activate RyR to cause CICR (28). It is noteworthy that we used concentrations of pharmacological agents that produced less than maximal inhibition of their intended targets, so the observed degree of inhibition in rats represents a conservative estimate of the involvement of ADP ribosyl cyclase and RyR in the integrated renal responses.

Fig. 7. Effect of ryanodine receptor inhibition on renal vascular responses to ETB receptor stimulation by ET-1.  
A: average RBF change in response to intrarenal injection of sarafotoxin S6c under control conditions (bold line) and in the presence of ruthenium red infusion into the renal artery (fine line, 126 μg·kg−1·min−1).  
B: average RBF responses to S6c before (ctrl), during (ruth red), and after (rec) ruthenium red; n = 8. *P < 0.02.

Large systemic arteries predominantly express ETA receptors on VSMC and ETB receptors on endothelial cells (43). In many vascular beds, ETB receptors are also expressed on venous VSMC (37). In the kidney, ETA receptor stimulation on ETA receptors (36). It is noteworthy that another form of ADPR cyclase sensitive to retinoic acid may exist in VSMC, although this form is poorly characterized (8). In an earlier study, we reported weaker than normal acute renal vascular responses to ANG II and NE in CD38−/− mice, suggesting that CD38 exists in resistance arterioles and mediates vasoconstriction in the kidney triggered by AT1 and adrenoceptors (47). Presently, we found that mice deficient in CD38 show attenuated renal vascular responses to intravenous injection of ET-1, supporting the notion that ADPR cyclase mediates acute ET-1-induced renal vasoconstriction in the mouse.

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Fig. 8. Effect of ryanodine receptor inhibition on renal vascular responses to ETB receptor stimulation by ET-1. A: average RBF response to ET-1 injected intrarenally in the presence of BQ123 before (bold line) and during intrarenal nicotinamide infusion (6 mg·kg−1·min−1, fine line).  
B: average change in RBF response to ET-1 in the presence of BQ123 before (ctrl), during (nic), and after (rec) nicotinamide; n = 9. *P < 0.01.
VSMC produces vasoconstriction (26, 27). ETB receptor stimulation on endothelial cells leads to production of the vasodilator NO (27, 43), whereas ETB receptors on VSMC elicit vasoconstriction (11, 25, 26). This occurs both in vitro and in vivo (9, 11, 25, 30, 38). Since both ETA and ETB receptors mediate renal vascular responses to ET-1, we analyzed the contribution of the ADPR cyclase/RyR pathway to signaling by each receptor. Our experimental design included selective stimulation of each receptor subtype as well as both together. Antagonist specificity and dosage have been established previously in our laboratory (26, 27). Both BQ123 and BQ788 effectively produce near-complete inhibition of ETA and ETB receptor subtypes, respectively, at the doses employed herein. Our present results together with previous studies by Just et al. (26, 27) suggest that antagonist concentrations of ryanodine inhibited [Ca^{2+}]_{i} responses to ETA receptor stimulation (12). Similarly, the cADPR-selective antagonist 8NH_{2}-cADPR attenuated Ca^{2+} increases in response to ETB receptor stimulation in testicular peritubular smooth muscle cells (4). To our knowledge, only one study has examined the link between ETA receptor activation and RyR. This study of isolated peritubular smooth muscle cells showed that antagonist concentrations of ryanodine inhibited [Ca^{2+}]_{i} responses to ETA receptor stimulation (4). We provide new information

Nicotinamide attenuated renal vasoconstriction produced by ETA receptor stimulation by ET-1+BQ788, indicating a role for ADPR cyclase signaling in ETA receptor-induced renal vasoconstriction in vivo. Our previous study demonstrated a dilator effect on basal renal vascular resistance when a high dose of nicotinamide was administered (47). Importantly, the dose used in the present study was low enough to not affect resting RBF or MAP. Our in vivo results for vasomotor tone extend those for [Ca^{2+}]_{i} seen in isolated afferent arterioles (12) and testicular peritubular smooth muscle cells (4) and establish the importance of the ADPR cyclase signaling pathway in renal vascular responses in anesthetized rodents. In individual afferent arterioles, both nicotinamide and the cADPR-selective blocker 8Br-cADPR attenuated [Ca^{2+}]_{i} responses to ETA receptor stimulation (12). Similarly, the cADPR-selective antagonist 8NH_{2}-cADPR attenuated Ca^{2+} increases in response to ETB receptor stimulation in testicular peritubular smooth muscle cells (4). To our knowledge, only one study has examined the link between ETA receptor activation and RyR. This study of isolated peritubular smooth muscle cells showed that antagonist concentrations of ryanodine inhibited [Ca^{2+}]_{i} responses to ETA receptor stimulation (4). We provide new information

Fig. 9. Effect of nicotinamide inhibition of ADPR cyclase on ETB receptor-induced renal vasoconstriction during inhibition of nitric oxide production. A: RBF response to sarafotoxin S6c during N^\text{\textgamma}-nitro-L-arginine methyl ester (L-NAME) infusion before (bold line) and during nicotinamide infusion into the renal artery (6 mg·kg^{-1}·min^{-1}, fine line). B: change in RBF in response to S6c during L-NAME infusion before (ctrl), during (nic) and after (rec) nicotinamide; n = 8. *P < 0.005.

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Fig. 10. Attenuated renal vascular responses to ET-1 in mice lacking the ADPR cyclase CD38. A: RBF response to ET-1 in wild-type (WT; bold line) and CD38−/− (fine line) animals. B: maximum RBF responses to intravenous injection of ET-1 in WT control mice (open bar) and mice lacking CD38 (CD38−/−, closed bar); n = 9. *P < 0.02.
that RyR are important mediators of ET<sub>A</sub> receptor signaling in the renal vasculature in vivo. By design we focused on the role of ADPR cyclase and RyR in mediating ET-1-induced renal vasoconstriction and did not assess participation by other Ca<sup>2+</sup> signaling pathways. Interactions among multiple pathways are probable since cADPR has been shown to sensitize the RyR to [Ca<sup>2+</sup>], to favor CICR (17, 51, 52). For example, the [Ca<sup>2+</sup>], and renal vasoconstrictor response initiated by Ca<sup>2+</sup> entry through L-type Ca<sup>2+</sup> channels is potentiatab by basal RyR activity (14, 47).

We provide new evidence that the magnitude of constriction elicited by ET<sub>B</sub> receptors is dependent on the activity of the ADPR cyclase/RyR signaling pathway. Our results are consistent with those for [Ca<sup>2+</sup>], experiments in peritubular smooth muscle cells in vitro (4) and rat cerebellum where ET<sub>B</sub> receptor signaling is dependent on cADPR and/or RyR, but differ from previous findings in isolated afferent arterioles showing that [Ca<sup>2+</sup>], responses to S6c were not significantly diminished by nicotinamide (12). The contrast between results likely reflects differences between in vitro and in vivo preparations. Importantly, our study indicates that whereas ET<sub>B</sub> receptor responses may not be mediated by ADPR cyclase in afferent arterioles without intact endothelium in vitro, in vivo renal constrictor responses to ET<sub>B</sub> receptor activation are dependent on the enzyme.

Unlike ET<sub>A</sub> receptors that are only found on VSMCs, ET<sub>B</sub> receptors are present on both VSMCs and endothelial cells and contribute to both constrictor and dilator responses (43). Similarly, ADPR cyclase contributes to signaling pathways in both cell types (12, 41), a fact that may have an impact on the interpretation of our results. Since we have previously seen that high concentrations of nicotinamide can increase basal RBF (47) and since the vasoconstrictor response to ET<sub>B</sub> is reduced rather than enhanced by nicotinamide in the current study as would be expected if ET<sub>B</sub> receptor-induced dilation were inhibited, we conclude that the bulk of the activity of ADPR cyclase in response to ET-1 is likely in the VSMCs rather than endothelial cells. ADPR cyclase signaling may occur in renal endothelial cells. However, inhibition of ADPR cyclase suggests a predominant action in renal VSMC. The role of ADPR cyclase and RyRs in ET<sub>B</sub> receptor-induced vasodilatation in the renal microcirculation requires further investigation.

Interestingly, the attenuation of the ET<sub>B</sub> receptor agonist S6c-induced renal vasoconstriction by nicotinamide was significantly greater that of BQ123+ET-1 or BQ788+ET-1 (P < 0.05, data not shown). Synergy may exist between ETA and ET<sub>B</sub> receptor signaling pathways in the renal vasculature (25, 26). Our data likely reflect this idea, suggesting that ADPR cyclase is more important in ET<sub>B</sub> receptor signaling in the presence of active ET<sub>A</sub> receptors.

Since endothelial cells possess ET<sub>B</sub> receptors, we tested whether in vivo responses depend on ET<sub>B</sub> receptor-mediated NO production by investigating the contribution of ADPR cyclase to S6c-induced renal vasoconstriction during inhibition of synthase (NOS) activity. We found that inhibition of S6c responses by nicotinamide occurred in the presence of l-NAME, indicating involvement of ADPR cyclase independently of a functioning endothelium. As mentioned previously, the interactions between ADPR cyclase and NO appear to be complex and cell type specific. Our data clearly show that renal vasoconstriction elicited by ET<sub>B</sub> receptors occurs via ADPR cyclase activation regardless of whether NO is present and add to the aforementioned suggestion that the bulk of the effect of ADPR cyclase inhibition in the renal vasculature in vivo is dependent on VSMCs and not endothelial cells. In this regard, our results suggest that NO is probably not exerting a major inhibitory effect on ADPR cyclase activity in renal VSMCs in vivo as has been reported for coronary artery VSMCs in vitro (50).

In summary, we present RBF evidence showing that ADPR cyclase and RyR mediate acute renal vascular responses to ET-1 in vivo and extend our previous study of the renal microcirculation demonstrating the importance of the ADPR cyclase/RyR signaling pathway in G protein-coupled receptor stimulation by ANG II and NE (47). Ours is the first animal study to investigate this area in any vascular bed in vivo, where complex interactions between cell and tissue types occur naturally. Intrarenal infusion of nicotinamide or ruthenium red at doses that did not alter MAP or basal RBF attenuated RBF responses to ET-1. Importantly, this was the case for responses to selective ETA and ET<sub>B</sub> receptor stimulation as well. Moreover, CD38<sup>−/−</sup> mice showed attenuated RBF responses to ET-1. ET<sub>B</sub> receptor dependence on ADPR cyclase was seen even in the absence as well as the presence of NO production, suggesting direct effects on renal VSMC. Our results provide new insight into ET-1’s regulation of renal hemodynamics under normal conditions and have implications for regulation of renal hemodynamics in the hypertensive state, when ET receptors are upregulated and more tonically activated (42, 43).

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