Differential Effects of U46619 on Renal Regional Hemodynamics in the Rat: Involvement of Endothelin

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ABSTRACT

Many vasoactive agents produce qualitatively similar effects on blood flow in the renal cortex and medulla, evoking reductions or increases in blood flow in both regions. We demonstrated previously that endothelin-1 (ET-1) is an exception because it evoked an increase in medullary perfusion despite a potent cortical vasconstriction (Hercule and Oyekan, 2000). We report here that U46619 (11,9 epoxyprostaglandin H3), a selective agonist of prostaglandin H2 (PGH2)/thromboxane A2 (TXA2) (TP) receptor, evokes similar effects as ET-1. In the pentobarbital-anesthetized (60 mg/kg) rat, 1, 3, and 5 mg/kg U46619 dose dependently reduced mean arterial blood pressure by ~2 ± 4, ~8 ± 10, and ~31 ± 10 mm Hg, respectively; renal cortical blood flow (CBF) by ~50 ± 11, ~174 ± 45, and ~349 ± 43 perfusion units (PU), respectively; but increased medullary blood flow (MBF) by ~42 ± 16, ~51 ± 18, and ~61 ± 21 PU, respectively. Prostaglandin F2alpha, a TXA2 mimetic, produced similar effects as U46619. SQ29548 (1S-[1α,2α(Z), 3α,4α]-7-3[2-[phenylamino]carbonyl]hydrazino]-methyl-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (0.1 mg/kg), an antagonist of PGH2/TXA2 (TP), blunted U46619-induced hemodynamic changes without affecting that produced by phenylephrine. BMS182874 (5-([dimethylamino]-N-([3,4-dimethyl-5-isoxazoly]-lyd)-1-naphthalene sulfonamide) (40 mg/kg), an ETa-selective antagonist, blunted U46619-induced reduction in CBF by 54 ± 9% (p < 0.05) and the increase in MBF by 59 ± 18% (p < 0.05). Similarly, BQ788 (N-cis 2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-o-1-methoxy-carbonyltryptophanyl-o-norleucine) (1 mg/kg), an ETB-selective antagonist, blunted the effects of U46619 on CBF and MBF by 19 ± 3% (p < 0.05) and 48 ± 19% (p < 0.05), respectively. Combined administration of BMS182874 and BQ788 further attenuated U46619-induced reduction in CBF by 67 ± 8% (p < 0.05) and that on MBF by 61 ± 18% (p < 0.05). Phosphoramidon (10 mg/kg), an endothelin converting enzyme inhibitor, markedly blunted U46619-induced changes on CBF and MBF (p < 0.05). These findings are the first to demonstrate that U46619, through activation of ETa and ETb receptors, elicits renal cortical vasoconstriction and medullary vasodilation in the rat.

Interactions involving two or more hormonal systems are now widely recognized and well established between angiotensin II (AII), endothelin (ET-1), nitric oxide, and eicosanoids. Thus, eicosanoids are the putative mediators of the effects of many hormones, and a role was proposed for thromboxane A2 (TXA2) in the effects of ET because ET administration evoked increases in TXA2 production (Stier et al., 1992) and antagonism of prostaglandin (PG) H2/TXA2 (TP) receptors blunted ET-1-induced contraction of the aortic ring (Asano et al., 1994). In addition, ET receptor antagonism diminished endothelium-dependent contractions suggesting that endogenous ET may also regulate the release of PGH2 and TXA2 (Moreau et al., 1996). However, another layer of interaction exists between agonists at an upstream level preceding the involvement of eicosanoids. This is exemplified in the study in which angiotensin II infusion was not only associated with increased tissue ET synthesis but also can be reversed by an ETA receptor antagonist (Moreau et al., 1997). As further evidence of these interactions, acetylcholine-induced contraction of the rat aortic ring was attributed to endothelin-stimulated release of PGH2 and TXA2 through stimulation of ETA receptors (Moreau et al., 1996). Related to these observations is the demonstration that bosentan, a mixed ETA and ETB receptor blocker, antagonized directly the stimulation of TP receptors in the rat aorta (Moreau et al., 1996) and counteracted the effects of TXA2 at the receptor level in endothin-induced pulmonary hypertension in sheep (Snapper et al., 1998). These seminal observations suggest a

ABBREVIATIONS: AII, angiotensin II; ET, endothelin; TXA2, thromboxane A2; PGH2, prostaglandin H2; TP, PGH2/TXA2; MABP, mean arterial blood pressure; CBF, cortical blood flow; PE, phenylephrine; PU, perfusion units; MBBF, medullary blood flow; CBFV, cortical vascular resistance; MVR, medullary vascular resistance; U46619, 11,9 epoxyprostaglandin H3; SQ29548, [1S-[1α,2α(Z), 3α,4α]-7-[3-[2-[phenylamino]carbonyl]-[hydrazino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; BMS182874, 5-([dimethylamino]-N-([3,4-dimethyl-5-isoxazolyl]-1-naphthalene sulfonamide; BQ788, N-cis 2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-o-1-methoxy-carbonyltryptophanyl-o-norleucine;
unique interaction between ET and TP receptors and are supported by the demonstration that treatment of human cerebromicrovascular endothelial cells with U46619 (11.9 epoxymethano-prostaglandin H₂), a TP agonist, led to an increased production of ET-1 (Spatz et al., 1994; Yakubu and Leffler, 1999).

In evaluating renal hemodynamic changes in response to various agonists, we observed a unique increase in mediulary perfusion to U46619 but a strong cortical vasoconstriction, a response hitherto observed only with ET-1 (Gurbanov et al., 1996; Hercule and Oyekan, 2000). Therefore, these experiments tested the hypothesis that the disparate effects of U46619 on renal regional hemodynamics involve ET-1 production and/or activation of ET receptors. Our findings demonstrate that ETₐ and ETₐ receptors are involved in the hemodynamic effects of U46619 in the rat.

Materials and Methods

ET-1 and big ET-1 (Peninsula Laboratories, Belmont, CA) were stored in 0.1% acetic acid at -20°C. U46619 and PGF⁺⁰⁻ were obtained from Sigma Chemical Co. (St. Louis, MO) and initially dissolved in absolute ethanol, 1 mg/ml, diluted in normal saline to 50 µg/ml, and stored frozen in aliquots at -70°C. BMS182874 [5-(dime-thylamino)-N-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide], a gift from Dr. Stier (Department of Pharmacology, New York Medical College, New York), was dissolved in 0.1 M NaHCO₃ and pH adjusted to 7.6. BQ788 (Peninsula Laboratories) was dissolved in 25% DMSO. Phosphoramidon (Sigma), a prototype endothelin-converting enzyme inhibitor, was dissolved in normal saline. SQ29548 (Cyanam Chemical Co., Ann Arbor, MI) was dissolved in absolute ethanol and diluted in normal saline.

The experiments were performed on male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA; body weight 309 ± 7 g). The animals were maintained on standard rat food (Purina Chow; Purina, St. Louis, MO) and were allowed ad libitum access to water and food until the beginning of the experiments.

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and placed on a heated platform to maintain body temperature at 37°C. A tracheostomy (polyethylen 250) was performed for spontaneous ventilation, and a tail vein was cannulated with a 23G butterfly needle (Abbott Hospitals Inc., North Chicago, IL) for drug administration. A catheter (polyethylene 10) was implanted in the carotid artery and connected to a pressure transducer for recording mean arterial blood pressure (MABP). Regional blood flow was determined as described previously (Hercule and Oyekan, 2000). Briefly, a left laparotomy was performed and a pressure catheter (Periflux System 5000 version 1.20; Perimed, Stockholm, Sweden) was obtained as perfusion units (PU) and expressed as volts (100 U corresponding to 1 V). Cortical and medullary vascular resistance (CVR and MVR) were calculated as ratios of MABP to CBF and MBF (mm Hg/perfusion units).

Experimental Protocol. After surgery and placing of probes for recording regional blood flows, a 30- to 45-min equilibration period was allowed after which a dose-response relationship was established to U46619 (1, 3, and 5 or 10 µg/kg). Responses to big ET-1 (2.0 µg/kg), precursor of ET-1; PGF⁺⁰⁻ (0.3 and 1.0 µg/kg), a Txₐ mimic; and phenylephrine (PE, 10 µg/kg), a negative control, were also evaluated. These doses were given randomly by bolus intravenous injection. The rat was allowed to recover fully from the effect of one dose before another dose was given. After the responses to the last dose of U46619 were tested, an inhibitor/antagonist or its vehicle was administered and responses to U46619 or the other agonists were reestablished after 5 min. In time controls (n = 5), responses to U46619 obtained 1 h after the equilibration period were repeated 45 min later.

The effects of U46619 on regional blood flows were studied in the presence of SQ29548, a TP receptor antagonist (0.1 mg/kg i.v.; n = 4); BMS182874, an ETₐ receptor antagonist (40 mg/kg i.v.; n = 5); BQ788, an ETₐ receptor antagonist (0.5 mg/kg i.v., n = 5); combination of BMS182874 and BQ788 (n = 5–7); phosphoramidon (10 mg/kg i.v., n = 6); or their respective vehicles: 0.1 M NaHCO₃ for BMS182874, 25% DMSO for BQ788, 5% ethanol for SQ29548, and normal saline for phosphoramidon. In all cases, changes in CBF and MBF were continuously monitored. The doses of BMS182874 and BQ788 used were those that we used in our previous studies to effectively antagonize ET-1 and/or ET-3 renal hemodynamic responses (Hercule and Oyekan, 2000). The effects of the inhibitors/antagonists on U46619-induced changes in hemodynamics were evaluated by comparing renal effects with U46619, big ET-1, or PE before and after the administration of the inhibitors/antagonists.

Data Analysis. All responses were recorded as changes (Δ) relative to preinjection values and data expressed as mean ± S.E. Analysis of variance was used to compare dose-response curves between controls (vehicle-treated) and treated groups followed by Newman-Keuls test. In all cases, p < 0.05 was considered significant.

Results

Effect of U46619 on Renal Cortical and Medullary Blood Flows. Basal MABP, CBF, MBF, CVR, and MVR in pentobarbital-anesthetized (n = 11) rats were 113 ± 5 mm Hg, 485 ± 33 PU, 138 ± 8 PU, 0.24 ± 0.03 mm Hg/PU, and 0.88 ± 0.12 mm Hg/PU, respectively. Figure 1 shows a representative tracing of the response to U46619, PGF₂α, or phenylephrine on CBF and MBF. U46619 and PGF₂α evoked reductions in CBF but increases in MBF, whereas phenylephrine elicited reductions in both CBF and MBF. Figures 2 and 3 show that 1, 3, and 10 µg/kg U46619 (threshold dose, 300 ng/kg) reduced CBF (50–349 PU) and increased MBF (42–61 PU) in a dose-dependent manner. Similarly, PGF₂α, at doses of 0.3 and 1 µg/kg, produced effects qualitatively similar to those of U46619 (Fig. 2). Despite its consis-
CVR and MVR were unchanged in the presence of BQ788 (Table 2). Combined administration of BMS182874 and BQ788 evoked hemodynamic changes that were less than those produced by either BMS182874 or BQ788. Figure 3 shows that BMS182874 not only attenuated U46619-induced reduction in CBF (54 ± 9%; p < 0.05, n = 6) but also attenuated the increase in MBF (59 ± 18%; p < 0.05, n = 6). Similarly, BQ788 produced a slight but significant attenuation of the reduction produced by U46619 in CBF (19 ± 3%; p < 0.05, n = 5) and markedly attenuated the increase in MBF by 48 ± 19% (p < 0.05; n = 4). Combined administration of BMS182874 and BQ788 (n = 5–7) further attenuated the effects of U46619 on CBF and MBF by 67 ± 8% (p < 0.05) and 61 ± 18% (p < 0.05), respectively. Phosphoramidon (10 mg/kg) blunted the effects of big ET-1 (2 μg/kg) on CBF and MBF by 74 ± 10 and 77 ± 4%, respectively (p < 0.05). Similarly, phosphoramidon blunted U46619-induced reduction in CBF by 34 ± 6% (p < 0.05, n = 6) and the increase in MBF by 65 ± 10% (p < 0.05, n = 4) (Fig. 4) without affecting responses to PE (CBF, −139 ± 28 [control] versus −124 ± 14 PU [experimental]; MBF, −31 ± 8 [control] versus −27 ± 4 PU [experimental]).

**Discussion**

The findings in this study demonstrate two major novel observations, namely, U46619 evokes differential effects on renal hemodynamics and that ETs are involved in these effects. These findings are based on the following observations: 1) U46619 dose dependently increased MBF despite a potent renal vasoconstriction; 2) PGF_{2α} produced similar effects as U46619; 3) ET_{A} and/or ET_{B} receptor antagonism blunted the cortical and medullary hemodynamic effects of
U46619; and 4) inhibition of ET production attenuated U46619-induced changes on renal hemodynamics.

A two-way interaction between humoral factors in the regulation of vasomotor tone and renal function is an area of growing interest. A typical interaction exists between AII and ET-1 because AII potently stimulates ET-1 synthesis/release (Scott-Burden et al., 1991), and ET-1 dose dependently increased AII production by an enalapril-sensitive mechanism (Kawaguchi et al., 1990, 1991). A two-way interaction has been demonstrated between ET and U46619. Thus, ETs increase the production of TxA2 in the kidney (Stier et al., 1992) and may regulate the release of PGH2 and TxA2 in blood vessels (Moreau et al., 1996). On the other hand, U46619 was demonstrated to stimulate ET-1 production in cultured endothelial cells (Spatz et al., 1994; Yakubu and Leffler, 1999), and blockade of TP receptors attenuated ET-1-induced contraction of the aortic ring (Asano et al., 1994) just as blockade of ETα and ETβ receptors attenuated contraction of the rat aorta (Moreau et al., 1996). In the present study, the endothelin-like response to U46619 on renal regional hemodynamics reveals the possibility of a similar interaction between U46619 and ET as that obtained with AII (Maeso et al., 1997) and casts a role for ET receptors in U46619-induced changes in renal hemodynamics in the rat.

In this study, U46619 increased medullary blood flow but decreased cortical blood flow in every case. However, the effects of U46619 on blood pressure are complex as we observed decreases or increases in blood pressure and in some cases a biphasic effect. The latter was characterized by a pronounced initial fall followed by a brief increase in blood pressure. However, regardless of the blood pressure effect, the effects of U46619 on medullary and cortical blood flow were not changed, leading to increases in cortical vascular resistance but reductions in medullary vascular resistance. The hypertensive effect of U46619 is consistent with its in vitro vasoconstrictor effect. Although unusual, the hypotensive effect in this study is in agreement with an earlier study (Hui and Ogle, 1993), which demonstrated that U46619-induced hypotension was not due to a fall in cardiac output caused by pulmonary vasoconstriction nor due to a reflex from vagal afferents. A fall in blood pressure by U46619 may be caused by its ability to generate or release prostacyclin in vitro and in vivo (Mehta et al., 1984; Nicholson et al., 1984). Future studies will address the role of prostacyclin in U46619-induced renal hemodynamics.

The increase by U46619 in medullary perfusion despite a cortical vasoconstriction that was mimicked by PGF2α and the vasodepression by U46619 that was blunted by antagonism of TxA2 receptors with SQ29548 demonstrates that these effects involve activation of TxA2 receptors. The blunting of the vasodepression by U46619 following blockade of TP receptors is in agreement with the studies of Hui and Ogle (1993). TxA2 receptors in the kidney have been well described, and available information demonstrates that there are two subtypes—a vascular type found predominantly in glomerular mesangial cells (Abe et al., 1995) and a tubular subtype found in glomerular capillaries (Bresnahan et al., 1996). It does not appear that the differential effects to U46619 in the medulla versus the cortex are related to differential sensitivity of the receptor subtypes to U46619 because SQ29548 virtually abolished the effects of U46619 in
for increased ET-1 production in U46619 responses, we employed phosphoramidon that competitively blocked responses to big ET-1 and big ET-3 but not big ET-1 or ET-3 (Pollock et al., 1993). The attenuation by phosphoramidon of U46619-induced cortical vasoconstriction and medullary perfusion does not unequivocally demonstrate ET-1 production by U46619 but suggests that a background tone of endothelin is required for full expression of the effects of TP receptor activation on cortical and medullary renal blood flow in the rat.

In conclusion, this study demonstrated a role for endothelin in the activation of PGI2/TxA2 receptors leading to an ET_A- and ET_B-mediated cortical vasoconstriction but a predominant ET_B-mediated medullary vasodilatation in the rat.

References


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Fig. 4. Effect of phosphoramidon (10 mg/kg) on the reductions in CBF (upper panel) or increases in MBF produced by U46619 (1, 3, or 10 μg/kg) or big ET-1 (2 μg/kg) in the anesthetized rat. Data are presented as mean ± S.E.M. of the absolute changes in blood flow relative to preinfusion values. *p < 0.05 versus control.