Prostaglandin E_2 Abrogates Endothelin-induced Vasoconstriction in Renal Outer Medullary Descending Vasa Recta of the Rat

Erik P. Silldorff, Sai Yang, and Thomas L. Pallone
Department of Medicine, Division of Nephrology, Pennsylvania State University, Hershey Medical Center, Hershey, Pennsylvania 17033

Abstract

Endothelins (ET) and prostaglandin E_2 are synthesized in the inner medulla by collecting duct epithelium and interstitial cells, respectively. All ascending vasa recta (AVR) blood returns from the inner medulla to the cortex in outer medullary vascular bundles. We reasoned that hormones might influence medullary blood flow by diffusing across AVR fenestrations to modulate vasoconstriction of outer medullary descending vasa recta (OMDVR). To investigate this possibility, OMDVR dissected from vascular bundles were exposed to ET-1, 2, or 3. Each endothelin isoform induced stable vasoconstriction with potency, ET-1 > ET-2 > ET-3 (EC_{50}, 1.8×10^{-15}, 5.9×10^{-12}, and 8.8×10^{-10} M, respectively). The ET_A receptor antagonists BQ-123 and BQ-610 (10^{-6} M), as well as an ET_A and ET_B receptor antagonist combination, attenuated vasoconstriction due to ET-1 (10^{-12} M). BQ-123 had no effect on the response to ET-3 (10^{-8} M). The ET_B receptor antagonist BQ-788 (10^{-6} M) attenuated the response to ET-3 (10^{-10} M), but not that to ET-1 (10^{-12} M). Finally, PGE_2 (10^{-6} M) reversibly dilated OMDVR preconstricted with ET-1 (10^{-12} M) or ET-3 (10^{-8} M) but not ET-1 (10^{-10} M). We conclude that ET-1, 2, and 3 are potent constrictors of OMDVR and the response to ET-1 is mainly ET_A receptor subtype mediated, while ET-3 acts via the ET_B. PGE_2 modulates ET induced constriction. These findings are consistent with interactive feedback and control of medullary perfusion by locally synthesized hormones. (*J. Clin. Invest.* 1995. 95:2734–2740.) Key words: microcirculation • microperfusion • videomicroscopy • vasoconstriction • vasodilation

Introduction

Endothelins (ET)[1] are potent vasoactive peptides whose role in the control of the systemic and renal microcirculations continues to be elucidated (1, 2). In their investigations of the sites of endothelin production, Kitamura et al. found the renal medulla to have the highest concentrations of endothelins (3). Substantial evidence has shown that endothelins are synthesized in the renal medulla. Production of ET-1 and, to a lesser extent, ET-3 has been demonstrated in the collecting tubule (4–6). All blood flow to the renal medulla is derived from the efferent flow of juxtamedullary glomeruli (7) so that synthesis of endothelins by glomerular endothelial, mesangial (8), or epithelial cells (9) might also supply hormone to the medulla. Endothelins are known to exert a variety of effects including mitogenesis (10), modulation of sodium transport (11) and vasoconstriction (12). Additionally, ET stimulation of renal medullary interstitial cells leads to production of the abundant medullary eicosanoid, PGE_2 (13). PGE_2 is a renal vasodilator the blockade of which leads to diminution of medullary blood flow (14–16) and potentiation of ET-1–induced renal vasoconstriction (1).

The unique anatomical arrangement of the vasa recta suggests the existence of a feedback system for control of the total and regional distribution of medullary blood flow. Outer medullary descending vasa recta (OMDVR) are contractile microvessel segments (17, 18) that arise from efferent arterioles of juxtamedullary glomeruli and subsequently traverse the inner stripe of the outer medulla in tightly packed vascular bundles (19). Remarkably, the ascending vasa recta (AVR) in the bundles are only those which arise from the inner medulla. AVR derived from reanastomosis of the capillary plexus in the outer medullary interbundle region return to the cortex without rejoining vascular bundles (19). Based on this anatomical arrangement, we hypothesize that hormones (e.g., ET and PGE_2) produced in the inner medulla might modulate the contraction of pericytes on the ablumenal surface of adjacent vascular bundle descending vasa recta (DVR) by crossing the fenestrated AVR wall (20). Detailed studies of the permselective properties of AVR have not been performed, however, some experiments have indicated that macromolecules much larger than endothelins can gain access to the interstitium across AVR fenestrations (21–23).

These considerations and the recent demonstration by polymerase chain reaction of the mRNA encoding the ET_A and ET_B receptors in vascular bundles (24), have provided substantial motivation to perform a detailed investigation of the effect of the endothelin isoforms 1, 2, and 3 on OMDVR. These studies demonstrate that ETs are remarkably potent vasoconstrictors. Additionally, we have determined that PGE_2 can antagonize the effects of ET-1 and ET-3 in this important regulatory microvessel segment verifying that locally produced hormones can modulate OMDVR vasomotor tone.

Methods

In vitro microperfusion

Detailed descriptions of the methods employed to perfuse OMDVR (17) and document their contractility (18) have been provided. The method is a minor variation of that originally described by Burg (25) for the study of renal tubules. Vasa recta harvested from the outer

Address correspondence to Erik P. Silldorff, Dept. of Medicine, Division of Nephrology, Hershey Medical Center, P.O. Box 850, 500 University Dr., Hershey, PA 17033. Phone: 717-831-8156; FAX: 717-831-6776.

Received for publication 22 July 1994 and in revised form 1 February 1995.

1. *Abbreviations used in this paper:* AVR, ascending vasa recta; DVR, descending vasa recta; ET, endothelins; OMDVR, outer medullary descending vasa recta.

J. Clin. Invest.

0021-9738/95/06/2734/07 $2.00
Volume 95, June 1995, 2734–2740

medullary vascular bundles and perfused in vitro are exclusively OMDVR and not AVR which are also present in vascular bundles (17). In brief, young female Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were anesthetized by intraperitoneal injection of thiopental (50 mg/Kg) after which the kidneys were harvested, sliced and placed into cold (4°C) Hepes buffer (5 mM Hepes, 140 mM NaCl, 10 mM NaAcetate, 5 mM KCl, 1.2 mM $MgCl_2$, 1.71 mM Na_2HPO_4, 0.29 mM NaH_2HPO_4, 1 mM $CaCl_2$, 5 mM Alanine, 5 mM Glucose, and 0.5 g/dl Albumin, adjusted to pH 7.4 and bubbled with 100% O_2). Vasa recta were dissected from vascular bundles. The bundles were located by progressively peeling medullary tissue from the corticomedullary junction until vasa recta were encountered. Vasa recta are readily identifiable by their small diameter and irregular wall structure. One or two vessels were taken from each rat within 2 h of death.

The construction of pipettes has been described (17, 18). Holding pipettes have outside diameters of 12–15 μm and inner constrictions < 9 μm. Perfusion pipettes have outside diameters of ~ 6 μm. Collection pipettes were maintained in the range of 6–12 μm and interchanged to achieve an acceptable fit to the OMDVR segment under study.

Harvested vessels were transferred to the stage of an inverted microscope (Nikon diaphot) equipped with Differential Interference Contrast (DIC, Nomarski) optics. After cannulation, vessels were perfused at 37°C with the same Hepes buffer used for dissection. The bath solution was identical to the perfusate except for addition of hormones. The bath flow rate was 300 μl/min, delivered by a syringe pump. The addition of hormonal agents during experiments was achieved by three rapid exchanges of the bath from a prewarmed syringe. Micromanipulators, perfusion and collection apparatus and perfusion chamber were purchased from Instruments Technology and Machinery (San Antonio, TX). Temperature of the perfusion chamber was maintained at 37°C with a feedback system employing a CN9111A controller (Omega Engineering Inc., Stamford, CT).

Following a 30-min equilibration period, perfusions were standardized by the adjustment of collection rate to the desired value (5 nl/min in most experiments). Timed collections of vessel effluent were obtained with volumetric constriction pipettes after which collection rate was adjusted by changing the pressure in the perfusion pipette. Driving pressure, once adjusted to obtain 5 nl/min collection rate was maintained constant throughout each experiment. Continuous measurement of lumenal pressure in the microperfused vessel has not been feasible, however, we have performed separate experiments that show lumenal pressures to be less than 15 mmHg (18).

Videomicroscopy and measurement of vessel diameters

To evaluate the effects of vasoactive agents on OMDVR diameters, microperfusion experiments were recorded on video tape. The inverted microscope was equipped with a 20/80% beam splitter and a side port for attachment of a video camera (CCD model 72; Dage-MTI). During experimentation, OMDVR were observed with a 40× objective to yield a final magnification of 1,300 on the video screen. Experiments were recorded on a Panasonic model AG 1960 VCR with a microphone for audio recording of experimental events. During playback, vessel internal diameters were measured by calipers at the point of greatest constriction (following ET administration). This method has been demonstrated to yield data indistinguishable from that obtained by more cumbersome image analysis (18). Because of the sensitivity and intensity of the vasopressor response to endothelins, the edge detection algorithm of computer imaging software was, in many cases, unable to distinguish lumenal edges during near 100% constriction. The focus of the microscope and adjustment of the differential interference contrast optics were optimized to yield maximal contrast to the vessel wall. In this study, changes in vessel inner diameter are expressed as percent constriction, defined in terms of the basal diameter in the absence of hormones (Do) and the experimental diameter (D) by the expression, %Constriction $= (1 - D/Do) \times 100$.

Reagents

Endothelins 1, 2, and 3, BQ-123, BQ-610 and IRL-1038 were purchased from Peninsula Laboratories. RES-701-1 was obtained from Alexis and BQ-788 from American Peptide Company (Sunnyvale, CA). Indomethacin was purchased from Sigma. Peptides (except IRL-1038, RES-701-1, and BQ-788) were initially dissolved in deionized water. All pharmacological agents were solubilized and frozen at −20°C in small aliquots of 10^{-3}–10^{-5} M. Aliquots were thawed and diluted at least 1,000-fold on the day of the experiment. Unused portions were discarded. PGE_2 (Sigma) was dissolved in 100% ethanol and stored at −20°C at a concentration of 10^{-3}M. IRL-1038 was solubilized in 40–0.1% trifluoroacetic acid and 60% acetonitrile; RES-701-1 was dissolved in 15% DMSO and BQ-788 was dissolved in 100% methanol. Following $\geq$ 1,000-fold dilution of aliquots with buffer the solvents used did not produce vasoactive effects themselves nor did they affect the actions of added hormones.

Experimental protocol

Stability of OMDVR vasoconstriction by ET-1, 2, and 3. After cannulation, OMDVR were perfused for 30 min for equilibration. Subsequently ET-1, 2, or 3 (10^{-10} M) was added to the bath. Lumenal diameter measurements were taken every minute for the first 5 min and every 5 min thereafter for an additional 30 min. At 30 min ET was removed from the bath to observe recovery. After removal of hormone, recovery diameter measurements were taken every minute for 5 min and again at 10 min.

Concentration dependence of vasoconstriction by ET-1, 2, and 3. Following equilibration, ET-1, 2, or 3 was added to the bath in incremental concentrations. Due to their high potency ET-1 and ET-2 concentrations were increased from 10^{-20} M to 10^{-8} M by 100-fold increments. ET-3 was increased in the bath from 10^{-12} M to 10^{-7} M by 10-fold increments. Based upon the results of experiment 1 (see Results), 10 min were permitted to elapse following concentration changes before videorecording for diameter measurement.

The effects of transmural pressure on ET-1 potency in OMDVR were examined by generating truncated dose-response curves (10^{-16} M to 10^{-10} M) at differing collecting pressures (0 and 10 mmHg). Similarly, the effect of bath and perfusate albumin concentration (0.5 or 4.0 g/dl) on ET-1 potency was examined. 10 min were allowed between ET-1 concentration changes and measurements as previously described. Albumin at 0.5 g/dl was used in all other experimental procedures.

Attenuation of ET-1 and ET-3 induced vasoconstriction by ET_A and ET_B receptor antagonists. To determine the contribution of the ET_A receptor subtype to the responses of ET-1 and ET-3 we utilized the specific ET_A receptor antagonists, BQ-123 and BQ-610. Following control measurements, either BQ-123 or BQ-610 (10^{-6} M) was added to the bath for ten minutes to allow receptor binding and to determine possible agonistic activity. Next, ET-1 (10^{-12} M) or ET-3 (10^{-8} M) was added to the bath in the presence of antagonist. Finally, the antagonist was removed from the bath leaving ET alone. As a time control, changes in lumenal diameter in the absence of antagonists were also documented.

Similar experiments were conducted to determine the contribution of the ET_B receptor to the vasoconstrictor responses of ET-1 and ET-3 (a selective ET_B agonist). The ET_B receptor antagonists IRL-1038 (10^{-5} M), RES-701-1 (10^{-6} M), and BQ-788 (10^{-6} M) were added separately to the bath before and during the presence of ET-3 (10^{-8} M). In addition, the ability of BQ-788 (10^{-6} M) to attenuate ET-1 (10^{-10} M) induced constriction was assessed alone and in conjunction with BQ-123 (an ET_A antagonist) in a manner similar to that used for other antagonists. Control experiments were performed in the absence of antagonist.

Modulating effects of PGE_2 on ET-1– and ET-3–induced vasoconstriction. 10 min after hormone addition, OMDVR constricted with either ET-1 (10^{-10} M) or ET-3 (10^{-8} M) were videotaped for diameter measurement. Subsequently, 0.1% ethanol (the vehicle for PGE_2) was added to the bath and recording was repeated after five minutes. Next, PGE_2 (10^{-6} M) was added in the presence of ET-1 or ET-3. Diameters were again measured. Finally, PGE_2 and vehicle were removed from the bath leaving ET alone. Diameters were recorded after 10 min.

Several laboratories have documented a lack of effect of PGE_2 to

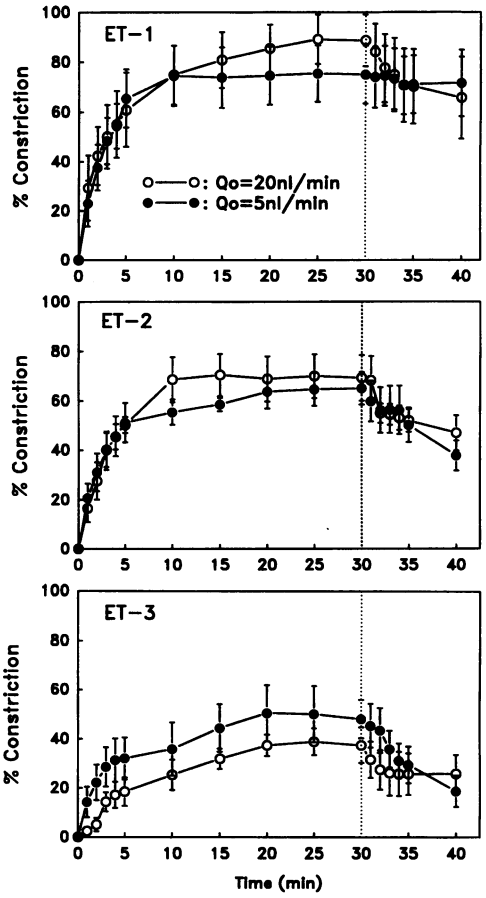

Figure 1. Time course of action for ET-1, 2, and 3. Percent constriction is shown as a function of time following addition of ET-1, 2, or 3 at 10^{-10} M (n = 12, 10, and 15, respectively). The vertical dotted line at 30 min represents the removal of ET from the bath. Initial collection rates of 5 (*solid circles*) or 20 (*open circles*) nl/min were established before addition of hormone. Data are means±SE.

abrogate severe renal vasoconstriction induced by high concentrations of ET-1. PGE_2 has, however, been effective at submaximal concentrations of ET-1 (1). Based on those observations, we tested the effect of PGE_2 on OMDVR preconstricted with 10^{-10} M ET-1 and 10^{-12} M ET-1.

To address the possible role of endogenous basal release of prostaglandins and/or ET-1–stimulated prostaglandins in our preparation we attempted to potentiate preexisting ET constriction (ET-1, 10^{-12} M) by adding indomethacin (10^{-6} M) to the bath solution. To assure that time-course of antagonist addition and prostaglandin halflife did not affect the results of acute indomethacin addition to OMDVR, a separate group of rats was injected with 5 mg/kg indomethacin (intraperitoneal) 30 min before i.p. sacrifice and subsequent exposure to ET-1 (10^{-12} M). Indomethacin (10^{-6} M) was also added to both the bath and perfusate. These results were compared to those obtained from sham injected rats.

Statistical analysis

Experimental results are reported as mean±SE. Statistical comparisons employ a paired t-test or repeated measures analysis of variance (ANOVA) as appropriate. For ANOVA, significance was determined by the Student-Newman-Keuls test. P values of less than 0.05 are considered significant.

Results

Stability of ET-1–, 2–, and 3–induced vasoconstriction. OMDVR exposed to ablumenal application of ET-1, 2, or 3

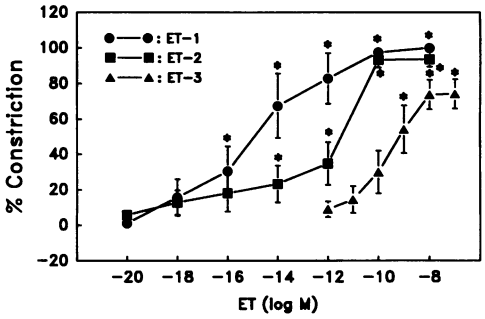

Figure 2. Concentration dependence of OMDVR vasoconstriction by ET-1, 2, and 3. Percent constriction is shown as a function of log-molar concentration of ET-1, ET-2, and ET-3 (n = 7, 9, and 7, respectively). *Significant change from OMDVR inner diameters prior to hormone addition ($P < 0.05$).

(10^{-10} M, n = 12, 10, or 15, respectively) constricted from a mean internal diameter of 9.9±2.2 μm to reach a minimum in ~ 10 min (Fig. 1). The constriction was stable for the duration of exposure to ET (30 min) and only moderately reversed 10 min following removal of ET from the bath. ET induced vasoconstriction was independent of initial perfusion rate (5 or 20 nl/min).

Concentration dependence of ET-1–, 2–, and 3–induced vasoconstriction. Significant concentration dependent vasoconstriction was observed with graded increase in concentration of all three ET isopeptides ($P < 0.05$, Fig. 2). By interpolation of the data, EC_{50} values for ET-1, 2, and 3 are, 1.8×10^{-15} M (n = 7), 5.9×10^{-12} M (n = 9), and 8.8×10^{-10} M (n = 7), respectively. The graded response to ET-1 is reproduced in a representative photomicrograph (Fig. 3). The vessel shown in Fig. 3 demonstrates diffuse constriction in response to ET-1,

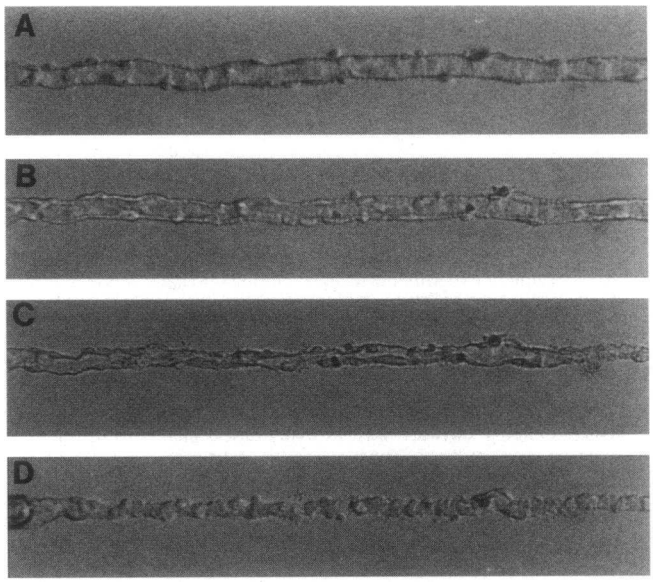

Figure 3. Representative photomicrograph of graded OMDVR vasoconstriction in response to ET-1. The same vessel photographed during control (*A*), 10^{-16} M (*B*), 10^{-14} M (*C*), and 10^{-12} M (*D*) ET-1. Diffuse constriction occurred in this vessel. Focal constriction was equally common in other OMDVR.

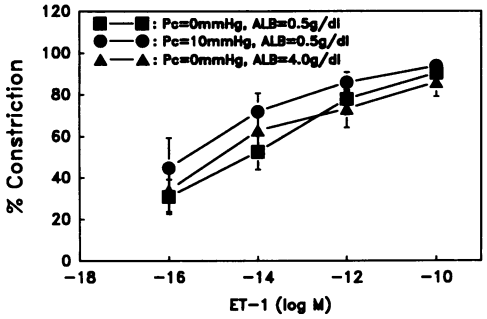

Figure 4. Effect of varying collection pressure (*Pc*) and albumin concentration on ET-1 vasoactivity. Truncated dose-response curves for ET-1 are shown with Pc = 0 mmHg and albumin = 0.5 g/dl (*squares*, $n = 7$), Pc = 10 mmHg and albumin = 0.5 g/dl (*circles*, $n = 6$), and Pc = 0 mmHg and albumin = 4.0 g/dl (*triangles*, $n = 7$). No significant differences are observed.

however, focal constriction at only a few locations along the vessel axis was equally common. Of the three isopeptides only ET-3 was unable to completely obliterate the lumenal space at maximally effective concentration.

Increasing collection pressure from 0 mmHg ($n = 7$) to 10 mmHg ($n = 6$) had no effect on the response of OMDVR to ET-1 (10^{-16} M to 10^{-10} M). Increasing albumin concentration from 0.5 to 4.0 g/dl ($n = 7$) was also without effect (Fig. 4).

Attenuation of ET-1– and ET-3–induced vasoconstriction by ET_A and ET_B receptor antagonists. The ET_A receptor antagonists BQ-123 and BQ-610 (10^{-6} M) significantly attenuated the response to ET-1 (10^{-10} M, $p < 0.05$, Fig. 5, $n = 5$ and 8, respectively), however, BQ-123 failed to attenuate the effect of ET-3 (10^{-8} M, Fig. 6, $n = 6$, BQ-610 not tested). The ET_B receptor antagonist BQ-788 (10^{-6} M) was tested alone ($n = 8$) and in conjunction ($n = 10$) with an ET_A receptor antagonist (BQ-123, 10^{-6} M) to determine the possible contribution of ET_B receptor stimulation to the ET-1 response. In both cases

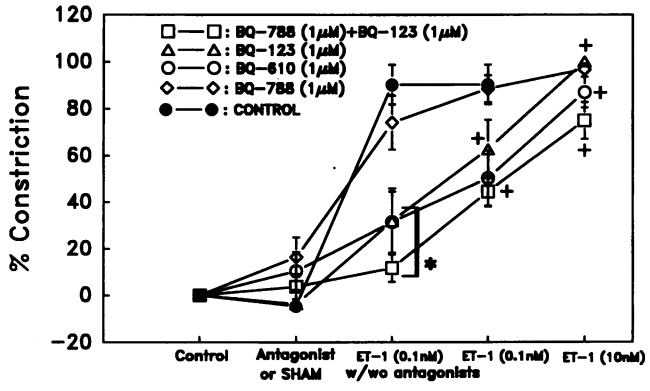

Figure 5. Functional antagonism of ET-1 by endothelin receptor antagonists. Antagonism of ET-1 mediated vasoconstriction by BQ-123 (*open triangles*, $n = 5$), BQ-610 (*open circles*, $n = 8$), BQ-788 (*open diamonds*, $n = 8$), and BQ-788 and BQ-123 (*open squares*, $n = 10$) is shown along with time controls in which no antagonist was added (*closed circles*, $n = 4$). Blockade of ET-1–induced vasoconstriction occurred with BQ-123, BQ-610, and BQ-123 + BQ-788 ($*P < 0.05$). Significant constriction by ET-1 occurred following removal of these same antagonists ($+ P < 0.05$).

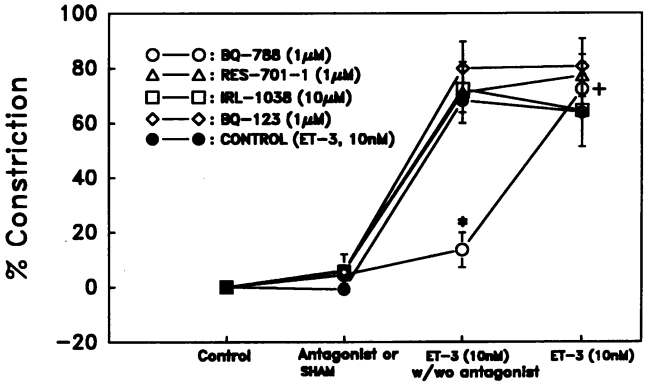

Figure 6. Functional antagonism of ET-3 by endothelin receptor antagonists. Percent constriction induced by ET-3 alone (*solid circles*, $n = 6$) and with BQ-123 (*open diamonds*, $n = 6$), IRL-1038 (*open squares*, $n = 5$), RES-701-1 (*open triangles*, $n = 6$) or BQ-788 (*open circles*, $n = 7$). Blockade of constriction occurring with BQ-788 ($*P < 0.05$) was followed by recovery ($+P < 0.05$).

BQ-788 tended to attenuate ET-1–induced constriction but the decrease was not statistically significant (Fig. 5).

To verify the ability of the vessels to respond to ET-1 following effective blockade, antagonist(s) were subsequently removed leaving ET-1 (10^{-10} M) in the bath. Following the removal of antagonist(s), further ET-1 induced constriction occurred ($P < 0.05$) but the recovery was not complete, suggesting a high affinity of the antagonist (s) for the receptor(s) (Fig. 5).

The ET_B receptor antagonists IRL-1038 (10^{-5} M) and RES-701-1 (10^{-6} M) as well as the ET_A receptor antagonist, BQ-123 (10^{-6} M) failed to attenuate the response to ET-3 (10^{-8} M, Fig. 6, $n = 5$, 6, and 6, respectively). In contrast, the ET_B receptor antagonist BQ-788 (10^{-6} M) significantly diminished the ET-3 response (Fig. 6, $n = 7$). The antagonists did not alter baseline diameters significantly when administered alone (Figs. 5 and 6).

Modulating effects of PGE_2 on ET-1– and ET-3–induced vasoconstriction. Ablumenal application of PGE_2 (10^{-6} M) to vessels preconstricted with ET-1 (10^{-10} M, $n = 6$) had no vasodilatory effect (Fig. 7). However, vessels preconstricted by a lesser concentration of ET-1 (10^{-12} M, $n = 8$) relaxed by 65% within 5 min of PGE_2 application ($P < 0.05$, Fig. 7). Vessels preconstricted with ET-3 (10^{-8} M, $n = 9$) were similarly dilated (63%) in the presence of PGE_2. Removal of PGE_2 from the bath leaving ET-1 or ET-3 alone resulted in significant recovery of constriction. PGE_2 was added to the bath by 1,000-fold dilution of 10^{-3} M stock dissolved in ethanol. Vehicle alone (ethanol) had no effect on vessels preconstricted with either ET-1 or ET-3 (Fig. 7).

Endothelin-1 (10^{-12} M) induced constriction was not altered by the addition of indomethacin (10^{-6} M) to the bath ($n = 8$, Fig. 8). Similarly, constriction was unaffected in OMDVR of rats pretreated with indomethacin (5 mg/kg, $n = 8$) when compared to diluent injected rats ($n = 7$, data not shown).

Discussion

Blood flow to the renal inner and outer medulla is derived exclusively from the efferent arterioles of juxtamedullary glo-

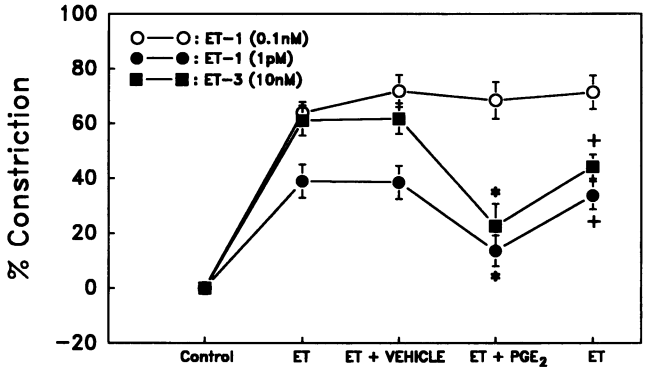

Figure 7. Modulating effects of PGE_2 on ET-1– and ET-3–induced vasoconstriction. ET-1 at 10^{-10} M (*open circles, n* = 6) and 10^{-12} M (*closed circles, n* = 8) and ET-3 (*closed squares, n* = 9) before, during, and after addition of 10^{-6} M PGE_2 to the bath. Significant vasodilation ($*P < 0.05$) and recovery of constriction ($+P < 0.05$) were observed with ET-3 and ET-1 (10^{-12} M).

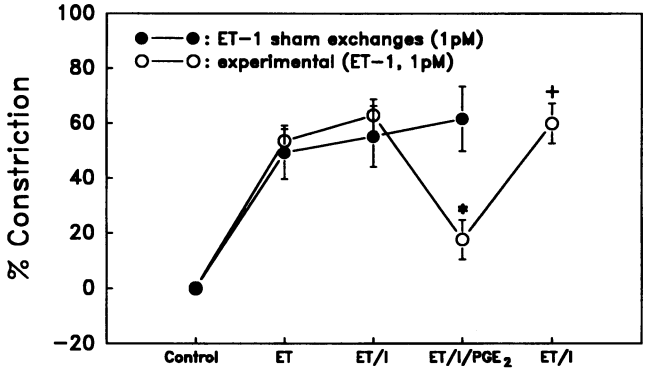

Figure 8. Lack of endogenous prostaglandin effects in ET-1 treated OMDVR. Indomethacin (I) effects on ET-1–induced constriction and PGE_2 dilation were examined (*open circles, n* = 8) and compared to untreated OMDVR (*n* = 7). Significant vasodilation ($*P < 0.05$) occurred with PGE_2, followed by recovery of constriction following removal ($+P < 0.05$).

meruli. Aglomerular routes have largely been discounted (19). In the outer stripe of the outer medulla, juxtamedullary efferent arterioles give rise to OMDVR all of which traverse the inner stripe in vascular bundles. Vascular bundles contain OMDVR, AVR returning from the inner medulla and, depending upon the species, the thin descending limbs of short looped nephrons (19). Thus, OMDVR perfuse the entire medulla and are exposed to AVR blood returning from the inner but not the outer medulla of the kidney. OMDVR on the bundle periphery give rise to the capillary plexus that perfuses nephron segments in the outer medullary interbundle region of the inner stripe while those in the bundle center cross the inner–outer medullary junction to supply the inner medulla (19, 26).

In the present context, two features of this microanatomical arrangement are particularly relevant. First, endothelin produced in the inner medulla can reach pericytes on the ablumenal surface of OMDVR by diffusing across the walls of AVR in vascular bundles. Second, if radial variation of OMDVR responsiveness to endothelin exists within the bundles, endothelins might play a role to determine the fraction of juxtamedullary efferent flow that perfuses the outer vs inner medulla of the kidney. An integral assumption is that the AVR wall is permeable to endothelin (mol wt = 2492). Detailed investigations of AVR permselectivity have not been performed, however, histochemical tracers as large as catalase and ferritin have been observed to cross AVR fenestrations (22, 23) and the reflection coefficient of AVR to albumin is less than 1.0 (21). ET-1 and ET-3 produced by the inner medullary collecting duct probably diffuses into adjacent inner medullary AVR plasma. Recently, we found significant permeation of OMDVR by inulin (Pallone, T. L., S. Nielsen, E. P. Silldorff, and S. Yang, manuscript submitted for publication) so that endothelins might also be expected to enter DVR plasma. Based upon these considerations it seems reasonable to suggest that countercurrent exchange and trapping of endothelin in the renal medulla could occur.

The generation of endothelins in the inner medulla may exert diuretic and natriuretic effects through direct stimulation of tubular cells (1). Additionally, endothelins stimulate medullary interstitial cells to produce PGE_2 (13), an autacoid which has been shown to decrease sodium reabsorption by the thick ascending limb of Henle (27) and the collecting tubule (28). The ability of endothelins to constrict OMDVR might act to abrogate these primary and secondary saluretic stimuli. Increases in renal perfusion pressure are known to induce natriuresis (termed pressure natriuresis). The detailed mechanisms responsible for pressure natriuresis have not been fully delineated. However, based on results showing that increases in medullary interstitial pressure and pressure natriuresis are jointly eliminated by renal decapsulation (29), increases in vasa recta hydraulic pressure and medullary interstitial pressure appear to be primary events. Through their ability to constrict OMDVR, endothelins might blunt increases in medullary microcirculatory and interstitial hydraulic pressure thus reducing the ability of the kidney to undergo pressure natriuresis. The net effect on renal sodium handling of the tubular and vascular actions of endothelins is difficult to predict.

This is the first study to demonstrate that ET-1, 2, and 3 vasoconstrict outer medullary descending vasa recta (OMDVR). In view of the critical location of these vessels and the high production (1) and concentration of endothelins in the renal medulla (3), pathophysiological implications may exist. Brezis and colleagues have demonstrated that the highly metabolic thick ascending limb of Henles loop is particularly vulnerable to ischemic insult (30). The medullary portion of the thick ascending limb of Henles loop resides in the interbundle region of the outer medulla and is therefore supplied with nutrients and oxygen by OMDVR on the vascular bundle periphery. Marked vasoconstriction of those vessels by endothelins or other hormones might play a role in the induction or exacerbation of ischemic acute renal failure. In support of this hypothesis, Chan et al. and Gellai et al. demonstrated abrogation of ischemic acute renal failure in rats treated with the ET_A receptor blocker, BQ-123 (31, 32).

Recognizing this is the first study of the actions of endothelins on OMDVR, we meticulously characterized the effect of each isopeptide and the receptor subtypes which mediate vasoconstriction. Maximal vasoconstriction occurs within minutes after application of endothelins (Fig. 1) and these hormones have a remarkable potency (threshold effect 10^{-18} M, Fig. 2). It should be recognized, however, that our observations with the in vitro perfused OMDVR provides only a system for bioassay of concentration dependent hormonal effects on vasocon-

striction. As such, the intense vasoconstriction observed in vitro might not exist in vivo even if local endothelin concentrations are as high as those employed in our experiments. The absence of modulating vasodilators probably accentuates the apparent response in the in vitro perfused microvessel preparation.

The threshold concentrations capable of eliciting OMDVR vasoconstriction in our preparation are well below typical plasma levels of immunoreactive endothelins. This implies, first, that endothelins could contribute significantly to basal OMDVR vasomotor tone in vivo and, second, that modulating endogenous vasodilators may play important roles to maintain and modulate renal medullary perfusion. In support of this concept, several authors (14–16) have shown that cyclooxygenase inhibition diminishes medullary blood flow in vivo.

We demonstrated that ET-1, at least in part, acts on OMDVR through stimulation of an ET_A receptor subtype while ET-3 appears to act through the ET_B receptor (Figs. 5 and 6). It is generally accepted that ET-1 activates ET_A or ET_B while ET-3 action occurs by stimulation of ET_B alone. The ET_B receptor subtype has been localized to the vascular endothelium where it mediates the secretion of nitric oxide (33) and to smooth muscle where it mediates vasoconstriction (12). The inability to attenuate the response of ET-3 with either IRL-1038 or RES-701-1 may indicate a lack of affinity or specificity of these antagonists for the ET_B receptor subtype in the rat (34).

Vasoconstriction of the renal microvasculature by endothelins has previously been demonstrated by a number of authors. Edwards et al. showed that rabbit efferent and afferent arterioles constrict in response to ET-1 and ET-2 ($EC_{50} \sim 1$ nM) while ET-3 is significantly less potent (35). Similarly, in the hydronephrotic rat kidney both efferent and afferent arterioles constricted in response to topical or lumenal administration of ETs, however, the afferent arteriole may be more sensitive (36–38). Other investigators have demonstrated the ability of ET to alter glomerular filtration rate and renal vascular resistance in vivo (1).

Wilkes et al. demonstrated stimulation of PGE_2 production in renal medullary interstitial cells by ET-1, but not by ET-3 (13). This suggests the existence of a mechanism to modulate the actions of ET through opposition by PGE_2. In our studies, PGE_2 reversibly dilated OMDVR preconstricted with 10^{-12} M ET-1 or ET-3, but not 10^{-10} M ET-1. These results are consistent with the finding that cyclooxygenase inhibition potentiates mild to moderate, but not severe renal vasoconstriction induced by ET-1 (1). The differing sensitivities of ET-1– and ET-3–induced constriction to PGE_2 might imply that the mechanism of interaction between PGE_2 and the cellular signal generated by ET-1 and ET-3 are different. Our experiments using indomethacin in isolated OMDVR demonstrate that endogenous cyclooxygenase products do not significantly affect the potency of endothelin or that adequate substrate is lacking in this in vitro perfused preparation.

In summary, this study provides the first description of vasoconstriction of OMDVR by endothelins. All ET isopeptides are extremely potent and induce stable vasoconstrictive responses. The receptor mediating the response to ET-1 is primarily ET_A, while the ET-3 response is generated via the ET_B subtype. The known stimulation of PGE_2 production in the renal medulla by ET-1 (13), coupled with the ability of PGE_2 to modulate OMDVR vasoconstriction by ET-1 or ET-3 suggests that a system may exist for feedback regulation of medullary perfusion by these autacoids. That feedback system might well be influenced by other vasoactive agents that are present in the renal medulla.

Acknowledgments

This work was supported by National Institutes of Diabetes and Digestive and Kidney Diseases Grant DK42495 and a Grant-in-Aid from the American Heart Association. These studies were performed during the tenure of an Established Investigatorship of the American Heart Association (T. L. Pallone).

References

1. Kohan, D. E. 1993. Endothelins in the kidney: physiology and pathophysiology. *Am. J. Kid. Dis.* 22:493–510.

2. Simonson, M. S. 1993. Endothelins: Multifunctional renal peptides. *Physiol. Rev.* 73:375–411.

3. Kitamura, K., T. Tanaka, J. Kato, T. Eto, and K. Tanaka. 1989. Regional distribution of immunoreactive endothelin in porcine tissue: abundance in inner medulla of kidney. *Biochem. Biophys. Res. Commun.* 161:279–283.

4. Kohan, D. E., and F. T. J. Fiedorek. 1991. Endothelin synthesis by rat inner medullary collecting duct cells. *J. Am. Soc. Nephrol.* 2:150–155.

5. Ujiie, K., Y. Terada, H. Nonoguchi, M. Shinohara, K. Tomita, and F. Marumo. 1992. Messenger RNA expression and synthesis of endothelin-1 along rat nephron segments. *J. Clin. Invest.* 90:1043–1048.

6. Uchida, S., F. Takemoto, E. Ogata, and K. Kurokawa. 1992. Detection of endothelin-1 mRNA by RT-PCR in isolated rat renal tubules. *Biochem. Biophys. Res. Commun.* 188:108–113.

7. Moffat, D. B. 1975. The Mammalian Kidney. Cambridge Univ. Press, London. 47–73.

8. Kohan, D. E. 1992. Production of endothelin-1 by rat mesangial cells: regulation by tumor necrosis factor. *J. Lab. Clin. Med.* 119:477–484.

9. Kasinath, B. S., T. A. Fried, S. Davalath, and P. A. Marsden. 1992. Glomerular epithelial cells synthesize endothelin peptides. *Am. J. Physiol.* 141:279–283.

10. Simonson, M. S., S. Wann, P. Mene, G. R. Dubyak, M. Kester, Y. Nakazato, J. R. Sedor, and M. J. Dunn. 1989. Endothelin stimulates phospholipase C, Na/H exchange, c-*fos* expression, and mitogenesis in rat mesangial cells. *J. Clin. Invest.* 83:708–712.

11. Perico, N., R. P. Cornejo, A. Benigni, B. Malanchini, J. R. Ladny, and G. Remuzzi. 1991. Endothelin induces diuresis and natriuresis in the rat by acting on proximal tubular cells through a mechanism mediated by lipoxygenase products. *J. Am. Soc. Nephrol.* 2:57–69.

12. Summer, M. J., T. R. Cannon, J. W. Mundin, D. G. White, and I. A. Watts. 1992. Endothelin ETA and ETB receptors mediate vascular smooth muscle contraction. *Br. J. Pharmacol.* 107:858–860.

13. Wilkes, B. M., A. S. Ruston, P. Mento, E. Girardi, D. Hart, M. Vander Molen, R. Barnett, and E. P. Nord. 1991. Characterization of endothelin-1 receptor and signal transduction mechanisms in rat medullary interstitial cells. *Am. J. Physiol.* 29:F579–F589.

14. Solez, K., J. A. Fox, M. Miller, and R. H. Heptinstall. 1974. Effects of indomethacin on renal inner medullary plasma flow. *Prostaglandins.* 7:91–97.

15. Cupples, W. A., T. Sakai, and D. J. Marsh. 1985. Angiotensin II and prostaglandins in control of vasa recta blood flow. *Am. J. Physiol.* 254:F779–F784.

16. Lemley, K. V., S. L. Schmitt, C. Holliger, M. J. Dunn, C. R. Robertson, and R. L. Jamison. 1984. Prostaglandin synthesis inhibitors and vasa recta erythrocyte velocities in the rat. *Am. J. Physiol.* 247:F562–F567.

17. Pallone, T. L., J. Work, R. L. Myers, and R. L. Jamison. 1994. Transport of sodium and urea in outer medullary descending vasa recta. *J. Clin. Invest.* 93:212–222.

18. Pallone, T. L. 1994. Vasoconstriction of outer medullary vasa recta by angiotensin II is modulated by prostaglandin E_2. *Am. J. Physiol.* 266:F850–F857.

19. Lemley, K. V., and W. Kriz. 1987. Cycles and separations: the histotopography of the urinary concentrating process. *Kidney Int.* 31:538–548.

20. Schwartz, M. M., M. J. Karnovsky, and M. A. Venkatachalam. 1976. Ultrastructural differences between rat inner medullary descending and ascending vasa recta. *Lab. Invest.* 35:161–170.

21. Pallone, T. L. 1992. Molecular sieving of albumin by the ascending vasa recta wall. *J. Clin. Invest.* 90:30–34.

22. Venkatachalam, M. A., and M. J. Karnovsky. 1972. Extravascular protein in the renal medulla. *Lab. Invest.* 27:435–444.

23. Shimamura, T., and A. B. Morrison. 1973. Vascular permeability of the renal medullary vessels in the mouse and rat. *Am. J. Pathol.* 71:155–167.

24. Terada, Y., K. Tomita, H. Nonoguchi, and F. Marumo. 1992. Different localization of two types of endothelin receptor mRNA in microdissected rat nephron segments using reverse transcription and polymerase chain reaction assay. *J. Clin. Invest.* 90:107–112.

25. Burg, M. B. 1972. Perfusion of isolated renal tubules. *Yale J. Biol. Med.* 45:321–326.

26. Pallone, T. L., C. R. Robertson, and R. L. Jamison. 1990. Renal medullary microcirculation. *Physiol. Rev.* 70:885–920.

27. Stokes, J. B. 1979. Effect of prostaglandin E_2 on chloride transport across the thick ascending limb of Henle. Selective inhibition of the medullary portion. *J. Clin. Invest.* 64:495–502.

28. Stokes, J. B., and J. P. Kokko. 1977. Inhibition of sodium transport by prostaglandin E_2 across the isolated perfused collecting tubule. *J. Clin. Invest.* 25:1099–1104.

29. Roman, R. J., and A.-P. Zou. 1993. Influence of the renal medullary circulation on the control of sodium excretion. *Am. J. Physiol.* 265:R963–R973.

30. Brezis, M., S. Rosen, and P. Silva. 1984. Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. *J. Clin. Invest.* 73:182–190.

31. Chan, L., A. Chittinandana, J. I. Shapiro, P. F. Shanley, and R. W. Schrier. 1994. Effect of an endothelin-receptor antagonist on ischemic acute renal failure. *Am. J. Physiol.* 266:F135–F138.

32. Gellai, M., M. Jungus, T. Fletcher, R. DeWolf, and P. Nambi. 1994. Reversal of postischemic acute renal failure with a selective endothelin$_A$ receptor antagonist in the rat. *J. Clin. Invest.* 93:900–906.

33. De Nucci, G., R. Thomas, P. D'Orleans-Juste, E. Antunes, C. Walder, T. D. Warner, and J. R. Vane. 1988. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. USA.* 85:9797–9800.

34. Urade, Y., Y. Fujitani, K. Oda, T. Watakabe, I. Umemura, M. Takei, T. Okada, K. Sakata, and H. Karaki. 1994. Retraction concerning an endothelin B receptor-selective antagonist: IRL 1038, [Cys^{11}-Cys^{15}]-endothelin-1 (11-21). *FEBS (Fed. Eur. Biochem. Soc.) letters* 342:103.

35. Edwards, R. M., W. Trizna, and E. H. Ohlstein. 1990. Renal microvascular effects of endothelin. *Am. J. Physiol.* 259:F217–F221.

36. Loutzenhiser, R., M. Epstein, K. Hayashi, and C. Horton. 1990. Direct visualization of effects of endothelin on the renal microvasculature. *Am. J. Physiol.* 258:F61–F68.

37. Bloom, I. T. M., F. R. Bentley, M. A. Wilson, and R. N. Garrison, 1993. In vivo effects of endothelin on the renal microcirculation. *J. Surg. Res.* 54:274–280.

38. Fretschner, M., K. Endlich, E. Gulbins, R. E. Lang, K. Schlottmann, and M. Steinhausen. 1991. Effects of endothelin on the renal microcirculation of the split hydronephrotic rat kidney. *Renal Phys. Biochem.* 14:112–127.