Interactive modulation of renal myogenic autoregulation by nitric oxide and endothelin acting through ET-B receptors

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Shi Y, Lau C, Cupples WA. Interactive modulation of renal myogenic autoregulation by nitric oxide and endothelin acting through ET-B receptors. Am J Physiol Regul Integr Comp Physiol 292: R354–R361, 2007. First published September 21, 2006; doi:10.1152/ajpregu.00440.2006.—In rats, nitric oxide modulates renal autoregulation in steady-state experiments and the myogenic mechanism in dynamic studies. Interactive modulation of autoregulation by nitric oxide and endothelin-1, predominantly involving endothelin B receptors, has been reported although it remains unclear whether the interaction is synergistic or obligatory or whether it affects the myogenic component of autoregulation. Nonselective inhibition of nitric oxide synthase (L-NAME) with endothelin A and B selective receptor antagonists BQ-123 and BQ-788, all infused into the renal artery, plus time series analysis were used to test the interactive actions of nitric oxide and endothelin on renal vascular conductance and on autoregulation. Nonselective endothelin receptor antagonist blunted the constrictor response to subsequent L-NAME but had no effect on previously established L-NAME-induced vasoconstriction. BQ-123 did not affect conductance and caused only minor reduction in myogenic autoregulatory efficiency. Responses to BQ-123 and L-NAME were additive and not interactive. BQ-788 and L-NAME each caused strong vasoconstriction alone and in the presence of the other, indicating that coupling between nitric oxide- and endothelin B-mediated events is not obligatory. L-NAME augmented myogenic autoregulation, and subsequent BQ-788 did not alter this response. However, BQ-788 infused alone also enhanced myogenic autoregulation but resulted in significant impairment of myogenic autoregulation by subsequent L-NAME. Thus the interaction between nitric oxide and endothelin is clearly nonadditive and, because it is asymmetrical, cannot be explained simply by convergence on a common signal pathway. Instead one must postulate some degree of hierarchical organization and that nitric oxide acts downstream to endothelin B activation. Inhibition of nitric oxide synthases (NOS) by Nω-nitro-L-arginine derivatives causes an important and long-lasting rise in blood pressure accompanied by profound reduction of renal blood flow (RBF) (e.g., see Ref. 3) so that typically renal vascular conductance is reduced by 50 to 60%. It has been known for some time that nitric oxide is a strong modulator of renal autoregulation in rats. In classic steady-state pressure ramps, this appears as a left shift of the lower limit of autoregulation after NOS inhibition (24, 25, 32, 38). When single pressure steps were used to examine the kinetics of autoregulation, NOS inhibition enhanced the faster component of autoregulation (i.e., the myogenic mechanism) (17). In dynamic studies the signature of NOS inhibition includes reduction of coherence at frequencies < 0.08 Hz, increased slope of gain reduction by the myogenic mechanism plus increased amplitude of the associated phase peak, and usually, increased gain reduction in the frequency band from 0.05 to 0.28 Hz (8, 14, 17, 18, 40, 41). Although the left shift of the lower limit could result from the marked rise in efferent resistance (23, 40), other results are not susceptible to this explanation (5, 11, 12, 33). Together, these studies demonstrate that nitric oxide modulates the myogenic component of renal autoregulation, and they show that the modulation can be detected by several different experimental designs. It is tacitly assumed that the left shift seen in steady-state experiments during NOS inhibition reflects the increased autoregulatory efficiency displayed by the myogenic mechanism in dynamic studies. It is also presumed that the absence of significant change in gain in the steady-state experiment (e.g., see Refs. 2, 3, and 27) reflects the difficulty of demonstrating positive modulation of the already highly efficient autoregulation.

It has been reported that vascular responses to NOS inhibition are mediated by endothelin-1 (1, 31). Certain endothelin subtype B (ET-B) receptors on endothelial cells are coupled to endothelial NOS (37), although renal vascular smooth muscle has a substantial population of ET-B receptors that mediate vasoconstriction (20). Interactive modulation of steady-state renal autoregulation by endothelin-1, mediated by the ET-B receptor and by nitric oxide has been reported. Using the steady-state pressure ramp experiment in vivo, Kram et al. (25) explored potential interactions between endothelin-1 and nitric oxide with NOS inhibition plus ET-A and ET-B selective receptor blockers. As expected, the lower limit of autoregulation was left-shifted by Nω-nitro-L-arginine methyl ester (L-NAME). This response was abrogated by the nonselective endothelin antagonist bosentan but was not affected by blockade of ET-A alone. Blockade of ET-B receptors by BQ-788 had no effect on either the lower limit of autoregulation or on the efficiency of autoregulation at higher renal perfusion pressure. However, when L-NAME was administered in the presence of BQ-788, the efficiency of autoregulation was impaired and, in those animals in which a lower limit could be discerned, it was significantly right shifted. These results indicate a rather complex interaction between endothelin and nitric oxide, although they do not provide information concerning the location or mechanism of the interaction.
Both endothelin-1 and NOS inhibition (17, 18, 33, 40, 41) augment myogenic autoregulation in the renal circulation under circumstances in which the other agonist would not be expected to contribute substantially. In the hydronephrotic kidney, in vitro endothelin-1 has been demonstrated to augment myogenic autoregulation at concentrations below those required to cause vasoconstriction (22), although most studies show little effect of endogenous nitric oxide in this preparation (33, 36). In addition, there is recent evidence that neuronal NOS at the macula densa accounts for a substantial portion of the modulation of autoregulation by nitric oxide (14, 17, 33), and available evidence suggests that the macula densa is not a target of endothelin (35). Thus it is not clear how nitric oxide and endothelin-1 interactively modulate autoregulation.

Oddly, most previous studies of endothelin-nitric oxide interactions have compared NOS inhibition alone with NOS inhibition in the presence of endothelin receptor blockade. This limits the conclusions that can be drawn from these studies because, if endothelin were to mediate the effects of nitric oxide, prior endothelin receptor blockade should prevent responses to subsequent NOS inhibition. The present study used receptor subtype selective antagonists with bidirectional design and assessment of RBF dynamics to test the role of endothelin in renal vascular responses to nonselective NOS inhibition.

METHODS

This study was performed at the SMBD-Jewish General Hospital in Montréal and at the University of Victoria and was approved by the Animal Care Committees at both institutions, both operating under the guidelines promulgated by the Canadian Council on Animal Care.

Procedures. All experiments were performed in 10- to 14-wk-old male Wistar rats obtained from Charles River (Canada). Rats had free access to water and food at all times prior to experiments. Twenty minutes prior to anesthesia, each rat received buprenorphine (Temgesic, 0.01 mg/kg ip; Reckitt and Colman Pharmaceuticals, Wayne, NJ). Anesthesia was induced by 5% isoflurane in inspired gas (30% O₂-70% air). After induction, the anesthetic concentration was reduced to =2%. The animal was transferred to a servocontrolled, heated table to maintain body temperature at 37°C, intubated, and ventilated by a respirator (model RSP 1002; Kent Scientific, Litchfield, CT). During the 1-h postsurgical equilibration period, inspired anesthetic concentration was titrated to the minimum concentration that precluded a blood pressure response when the tail was pinched (=1%).

Cannulas were placed in the right femoral artery (PE-90 with narrowed tip) and vein (PE-50). A constant infusion delivered 1% body wt/h throughout the experiment and contained 2% charcoal-washed bovine serum albumin in normal saline. A fine Teflon cannula was placed in the left femoral artery and advanced into the abdominal aorta. The left kidney was approached by a flank incision, immobilized in a plastic cup, and covered with plastic wrap. The distal end of the Teflon cannula was manipulated into the renal artery after the artery was stripped from hilus to aorta. The flow probe (model 1PRB; driven by a Transonic Systems model T401 flowmeter) was placed around the renal artery; it was fixed in place, and acoustic coupling was assured by application of a gel based on surgical lubricant and NALCO 1181 as recommended (35a). Femoral arterial pressure was measured by a Kent pressure transducer (model TRN050) driven by a model TRN005 amplifier.

In autoregulation experiments, a motorized clamp was placed on the aorta between the right and left renal arteries and was used to force blood pressure. The motor was driven by a program implemented in DT-VEE (Data Translation), which operates as a negative-feedback controller to maintain downstream pressure 15–20% below the spontaneous level of blood pressure. It iterates at 2 Hz, and at each iteration the target pressure is randomly changed within a predefined range, typically ± 5% (42).

Experimental drugs were infused into the renal artery using the mixing pump reported by Parekh (30). Pilot experiments established that placing the cannula tip into the renal artery or operating the mixing pump had no discernible effect on spontaneous blood pressure or RBF.

Inhibition of NOS was achieved by L-NAME (Sigma) delivered at 10 µg/min × 20 min, then at 3 µg/min for the remainder of the experiment (33). The nonselective endothelin antagonist PD-145,065 (Sigma) was infused at a nominal concentration (assuming RBF = 5 ml/min and hematocrit = 0.4) of 75 nM (9), except as noted below. The endothelin subtype A receptor (ET-A) selective antagonist BQ-123 and the ET-B selective antagonist BQ-788 (Alexis Biochemicals) were each delivered at a nominal concentration of 1 µM (15, 16, 21, 29, 39). Preliminary experiments (n = 8) showed that intrarenal infusion of endothelin-1 (Sigma) at 5 ng·kg⁻¹·min⁻¹ reduced RBF by 5% and that this constriction was effectively blocked by PD-145,065 (ΔRBF = −3 ± 3%) and by combined infusion of BQ-123 and BQ-788 (ΔRBF = +1 ± 5%).

Experiments. If the effects of two compounds are additive, then the response to combined application will be independent of the order in which they are applied. Dependence of the final result on the sequence of application defines an interaction between the two (6). Consequently, in the three experiments described below, the initial control period was followed in some rats first by intrarenal infusion of L-NAME and then by L-NAME plus endothelin receptor antagonist, while in other rats the endothelin receptor antagonist was infused first and then L-NAME was added.

Experiment 1 tested whether the renal vasoconstrictor actions of nonselective endothelin inhibition and nonselective NOS inhibition are interactive. Because endothelin-1 dissociates slowly from its receptors, PD-145,065 was infused for at least 80 min before measurements were made. Eight rats received first L-NAME alone, then L-NAME plus PD-145,065 (75 nM). Eight rats received first PD-145,065, after which L-NAME was added to the infusate. RBF was measured at the end of each interval. In four rats PD-145,065 was infused at a nominal concentration of 75 nM in renal arterial blood and in four rats at 225 nM. No differences were apparent, and the results were pooled.

Experiment 2 examined the responses of RBF and of RBF dynamics to single and combined inhibition of NOS and of ET-A receptors. In 10 rats, the control period (saline vehicle) was followed sequentially by L-NAME and then by L-NAME plus BQ-123. In another seven rats, the control period was followed sequentially by BQ-123 and then by BQ-123 plus L-NAME. In each period, drugs were infused for 30 min and renal perfusion pressure was then forced for 25 min during continued infusion.

Experiment 3 examined the responses of renal vascular conductance and of RBF dynamics to single and combined inhibition of NOS and of ET-B receptors. In seven rats, the control period (saline vehicle) was followed by L-NAME and then by L-NAME plus BQ-788; in another six rats, the control period was followed by BQ-788 and then by BQ-788 plus L-NAME. In each period, drugs were infused for 30 min and renal perfusion pressure was then forced for 25 min.

Data acquisition and analysis. The blood pressure and RBF signals were low-pass filtered at 20 Hz and sampled at 100 Hz online. Data segments of 1311 s were band-pass filtered (0.004 and 1 Hz) by using a fast-Fourier transform (FFT)-inverse FFT filter with a rectangular window and subsampled to 3.125 Hz. Power spectra, transfer functions, and squared coherences based on the FFT were computed by using standard algorithms as described previously (33, 42), using 1024-point segments shaped by the Hann window. Coherence is an index of the linear relationship between the input and output; if coherence = 1, all variation in the output variable can be explained as...
a linear function of variation of the input, while coherence = 0 suggests that the signals are unrelated. Gain in decibels (dB) was calculated as $20 \times \log$ (admittance magnitude/conductance). When gain $> 0$ pressure fluctuations are amplified into blood flow as expected from passive, compliant vessels; flow is being stabilized when gain $< 0$. The slope of gain reduction by a control system and the height of the associated phase peak provide information about the internal dynamics of that system. A system that responds only to the level of the input variable will typically have a slope $= 20$ dB/decade and a phase peak of $\pi/4$ radians (rad) at the corner frequency, whereas a system that responds to both the level and the rate of change of the input variable will have a slope $= 40$ dB/decade and a phase peak of $\pi/2$ rad (18, 28). Slope was determined by least squares fitting of the linear region of gain reduction, determined by inspection; the phase peak was estimated as the average phase within the same region of the spectrum. Under control conditions in normotensive rats, the slope of gain reduction is typically in the range of 20–30 dB/decade (40).

In rats anesthetized by isoflurane, the myogenic system operates at $0.2$ Hz, while tubuloglomerular feedback operates at $0.03–0.05$ Hz (7, 41, 42). To assess the contribution of the myogenic system to stabilization of RBF, we determined fractional compensation that is calculated from gain: fractional compensation $= 1 - [10^{\text{gain}(\text{dB})}]$. Fractional compensation $= 1$ implies complete autoregulation and fractional compensation $= 0$ indicates that autoregulation is ineffective in the frequency interval from 0.05 to 0.08 Hz that was used to minimize corruption by tubuloglomerular feedback (< 0.05 Hz) and by myogenic transients (> 0.08 Hz) (42).

Treatment effects were assessed by two-way, repeated-measures ANOVA (Statistica, version 5.5) and completed by planned contrasts comparing between coincident periods in the two sequences within an experimental sequence and comparing consecutive periods within an experimental sequence and between the two experimental sequences. The discrete high-frequency peaks reflect respiratory signals from animals that breathed slowly. Coherences between renal perfusion pressure and RBF are shown in Fig. 1, B and F; coherence was high in control, was reduced in the low frequency band from 0.01 to 0.08 Hz by l-NAME in the absence ($P = 1 \times 10^{-5}$) or presence of BQ-123 ($P = 0.008$), and was unaffected by BQ-123 in the absence or presence of l-NAME. Admittance gains are shown in Fig. 1, C and G, and admittance phases are shown in Figs. 1, D and H. Variables extracted from these transfer functions (fractional compensation, slope of gain reduction, phase peak) are reported in Table 2. l-NAME alone (Fig. 1, C and D) increased the slope of gain reduction (shown by the dashed lines; $P = 5 \times 10^{-4}$) and the corresponding phase peak ($P = 5 \times 10^{-5}$), while l-NAME during BQ-123 infusion (Fig. 1, G and H) caused comparable increases in the slope ($P = 0.003$) and the phase peak ($P = 6 \times 10^{-7}$). L-NAME also increased the fractional compensation achieved by the myogenic mechanism, both alone ($P = 2 \times 10^{-5}$) and during BQ-123 infusion ($P = 8 \times 10^{-4}$). BQ-123 had lesser effects on the transfer function. When infused alone, it reduced fractional compensation ($P = 0.029$) but did not significantly alter the slope of gain reduction or the phase peak (both $P > 0.3$). When infused during l-NAME infusion, BQ-123 reduced fractional compensation ($P = 0.029$) but did not significantly alter the slope of gain reduction ($P > 0.4$) or the associated phase peak ($P > 0.2$).

Table 1. Responses of blood pressure and conductance to nonselective endothelin receptor blockade and inhibition of nitric oxide synthesis

<table>
<thead>
<tr>
<th>Control</th>
<th>l-NAME</th>
<th>PD-145065 During l-NAME</th>
<th>Control</th>
<th>PD-145065 During PD-145065</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure, mmHg</td>
<td>121±4</td>
<td>135±3*</td>
<td>134±2</td>
<td>110±4</td>
</tr>
<tr>
<td>Renal blood flow, ml/min</td>
<td>8.40±0.56</td>
<td>3.84±0.39†</td>
<td>3.98±0.42</td>
<td>8.94±0.89</td>
</tr>
<tr>
<td>Renal vascular conductance, ml-min⁻¹-mmHg⁻¹</td>
<td>0.070±0.005</td>
<td>0.029±0.003†</td>
<td>0.030±0.003</td>
<td>0.083±0.009</td>
</tr>
</tbody>
</table>

Data are reported as means ± SE. l-NAME, Nω-nitro-l-arginine methyl ester. Superscripts denote statistical significance: comparison with previous in the same sequence: *$P < 0.01$, †$P < 0.001$; comparison with the same period in the reverse protocol: a$P < 0.05$, b$P < 0.01$, c$P < 0.001$. 

Figure 1 presents the RBF dynamics acquired in the BQ-123 experiments. On the left (Fig. 1, A–D) are shown the data acquired when l-NAME was infused first and BQ-123 was added; data acquired when BQ-123 was infused first and l-NAME added are shown on the right (Fig. 1, E–H). Figure 1, A and E show the power spectra of renal perfusion pressure, which did not differ among periods and were very similar in the two sequences. The discrete high-frequency peaks reflect respiratory signals from animals that breathed slowly. Coherences between renal perfusion pressure and RBF are shown in Fig. 1, B and F; coherence was high in control, was reduced in the low frequency band from 0.01 to 0.08 Hz by l-NAME in the absence ($P = 1 \times 10^{-5}$) or presence of BQ-123 ($P = 0.008$), and was unaffected by BQ-123 in the absence or presence of l-NAME. Admittance gains are shown in Fig. 1, C and G, and admittance phases are shown in Figs. 1, D and H. Variables extracted from these transfer functions (fractional compensation, slope of gain reduction, phase peak) are reported in Table 2. l-NAME alone (Fig. 1, C and D) increased the slope of gain reduction (shown by the dashed lines; $P = 5 \times 10^{-4}$) and the corresponding phase peak ($P = 5 \times 10^{-5}$), while l-NAME during BQ-123 infusion (Fig. 1, G and H) caused comparable increases in the slope ($P = 0.003$) and the phase peak ($P = 6 \times 10^{-7}$). L-NAME also increased the fractional compensation achieved by the myogenic mechanism, both alone ($P = 2 \times 10^{-5}$) and during BQ-123 infusion ($P = 8 \times 10^{-4}$). BQ-123 had lesser effects on the transfer function. When infused alone, it reduced fractional compensation ($P = 0.029$) but did not significantly alter the slope of gain reduction or the phase peak (both $P > 0.3$). When infused during l-NAME infusion, BQ-123 reduced fractional compensation ($P = 0.029$) but did not significantly alter the slope of gain reduction ($P > 0.4$) or the associated phase peak ($P > 0.2$). Inspection of Fig. 1 and Table 2 indicates that the effects of l-NAME and BQ-123 on RBF dynamics are independent and additive.

Renal vascular responses to infusion of l-NAME and BQ-788 are presented in Table 3. Renal perfusion pressure was stable in the forward experiments (control to l-NAME to BQ-788 plus l-NAME), but in the reverse sequence, infusion of BQ-788 alone was associated with a rise of renal perfusion pressure that was maintained during subsequent l-NAME infusion. Both l-NAME and BQ-788 increased the slope of RBF gain reduction (Fig. 3), associated with increases in fractional compensation (Fig. 3).
and BQ-788 caused profound renal vasoconstriction, whether acting alone or in the presence of the other antagonist.

Figure 2 presents the RBF dynamics acquired in the BQ-788 experiments. On the left (Fig. 2, A–D) are shown the data acquired when L-NAME was infused first and BQ-788 was added; data acquired when BQ-788 was infused first and L-NAME added are shown on the right (Fig. 2, E–H). The power spectra of renal perfusion pressure were similar among periods and between the two sequences (Fig. 2, A and E). Coherences, shown in Fig. 2, B and F, were high in control and reduced in the low-frequency band from 0.01 to 0.08 Hz by L-NAME in the absence of BQ-788 (P < 0.001); coherence was unaffected by BQ-788 in the absence or presence of L-NAME. Admittance gains are shown in Figs. 2, C and G, and admittance phases are shown in Figs. 2, D and H. Variables extracted from these transfer functions are reported in Table 3. In the forward experiment (L-NAME first), the slope of gain reduction by the myogenic system (shown by the dashed lines) was high during the control period and consequently was

<table>
<thead>
<tr>
<th>Renal perfusion pressure, mmHg</th>
<th>Control</th>
<th>L-NAME</th>
<th>During L-NAME</th>
<th>Control</th>
<th>L-NAME</th>
<th>During L-NAME</th>
<th>Control</th>
<th>L-NAME</th>
<th>During L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow, ml/min</td>
<td>1.05 ± 0.98</td>
<td>3.27 ± 0.45</td>
<td>3.31 ± 0.51</td>
<td>5.75 ± 0.63</td>
<td>6.38 ± 0.75</td>
<td>3.32 ± 0.57</td>
<td>3.27 ± 0.45</td>
<td>3.31 ± 0.51</td>
<td>5.75 ± 0.63</td>
</tr>
<tr>
<td>Renal vascular conductance, ml/min⁻¹·mmHg⁻¹</td>
<td>0.064 ± 0.009</td>
<td>0.031 ± 0.004</td>
<td>0.032 ± 0.005</td>
<td>0.057 ± 0.008</td>
<td>0.068 ± 0.009</td>
<td>0.035 ± 0.007</td>
<td>0.064 ± 0.009</td>
<td>0.031 ± 0.004</td>
<td>0.032 ± 0.005</td>
</tr>
<tr>
<td>Fractional compensation, %</td>
<td>18 ± 7</td>
<td>51 ± 6‡</td>
<td>38 ± 9*</td>
<td>6 ± 8</td>
<td>6 ± 8</td>
<td>6 ± 8</td>
<td>18 ± 7</td>
<td>51 ± 6‡</td>
<td>38 ± 9*</td>
</tr>
<tr>
<td>Slope of gain reduction, dB/dec</td>
<td>22.0 ± 2.5</td>
<td>37.8 ± 2.9‡</td>
<td>35.3 ± 4.9</td>
<td>17.1 ± 3.5</td>
<td>14.1 ± 3.4‡</td>
<td>27.3 ± 5.3‡</td>
<td>22.0 ± 2.5</td>
<td>37.8 ± 2.9‡</td>
<td>35.3 ± 4.9</td>
</tr>
<tr>
<td>Phase peak, rad</td>
<td>0.82 ± 0.09</td>
<td>1.47 ± 0.12‡</td>
<td>1.33 ± 0.13†</td>
<td>0.76 ± 0.17</td>
<td>0.60 ± 0.12b</td>
<td>1.09 ± 0.16‡</td>
<td>0.82 ± 0.09</td>
<td>1.47 ± 0.12‡</td>
<td>1.33 ± 0.13†</td>
</tr>
</tbody>
</table>

Data are reported as means ± SE. Ten rats received L-NAME before BQ-123; 7 rats received BQ-123 first. dB/dec, decibels per decade. Superscripts denote statistical significance: comparison with previous period in the same sequence: *P < 0.05, †P < 0.01, ‡P < 0.001; comparison with the same period in the reverse sequence: *P < 0.01, †P < 0.001.
not affected by L-NAME or by subsequent BQ-788 (both \( P > 0.5 \)). L-NAME alone did, however, increase the phase peak (\( P = 0.007 \)), and BQ-788 had no further effect (\( P > 0.8 \)). Neither L-NAME (\( P = 0.082 \)) nor subsequent BQ-788 (\( P > 0.3 \)) significantly altered fractional compensation achieved by the myogenic mechanism. Although BQ-788 had little effect on RBF dynamics when administered during L-NAME infusion, it appeared to augment myogenic autoregulation when infused alone. As shown in Fig. 2H, the phase peak was increased (\( P = 0.03 \)), the slope of gain reduction was numerically, although not significantly, increased (\( P = 0.067 \)); fractional compensation also increased numerically, although not significantly (\( P = 0.065 \)). Subsequent L-NAME infusion reduced fractional compensation (\( P = 0.017 \)) and the slope of gain reduction (\( P = 0.017 \)) but did not significantly affect the phase peak (\( P = 0.3 \)).

**DISCUSSION**

The present study examined two renal vascular responses in which there is evidence for significant interaction between...
Nitric oxide and endothelin. There is a considerable literature showing that prior administration of endothelin receptor antagonists significantly inhibits, but does not block, the vasoconstrictive response to NOS inhibition (e.g., see Refs. 1, 25, and 31). This finding has been variably interpreted to indicate that endothelin mediates the renal vascular response to NOS inhibition (1, 31) or that there is a postreceptor interaction between the two signals pathways (25). We are aware of only one previous study examining potential interactions between endothelin and nitric oxide as modulators of renal autoregulation (25), although both are well-known modulators of the myogenic component of autoregulation (11, 18, 20, 22, 40, 41). The current results lead to two major conclusions. First, the effects on vascular tone of \( \text{L-NAME} \) and endothelin receptor blockade are consistent only with a postreceptor interaction. Second, the modulation of autoregulation that involves an interaction between nitric oxide and ET-B receptors, first reported by Kramp et al. (25), is shown to occur in the myogenic mechanism.

Previous studies showed that prior blockade of ET-A and ET-B receptors blunts the constriction induced by inhibition of NOS (1, 25, 31). A similar result is seen in the present study (Table 1). However, when PD-145,065 was infused after \( \text{L-NAME} \), there was no reversal of the constriction induced by \( \text{L-NAME} \). The dose of PD-145,065 employed was clearly adequate to prevent activation of ET receptors by exogenous endothelin-1 and was infused for ~90 min, presumably long enough to assure receptor occupancy by the antagonist. Failure to reverse the constriction induced by \( \text{L-NAME} \) indicates that endothelin cannot be a mediator of this response to NOS inhibition. Instead, this pattern is consistent with the presence of an interaction between nitric oxide and endothelin-1 at the postreceptor level (25).

Results from experiments 2 and 3 in which the endothelin receptor subtype selective antagonists BQ-123 (ET-A) and BQ-788 (ET-B) were used with \( \text{L-NAME} \) are also consistent with interactive effects. When infused alone, BQ-123 displayed, at most, a modest contribution to vascular tone. A similarly small and variable response was also seen when BQ-123 was infused during NOS inhibition, again consistent with a relatively minor ET-A-mediated contribution to renal vascular tone. In this experiment, the combined effects of \( \text{L-NAME} \) and BQ-123 were additive and provide no evidence for an interaction between nitric oxide and ET-A-mediated events. Schmidt et al. (34) have reported in humans that BQ-123 can partially reverse renal vasoconstriction due to nonselective NOS inhibition. The time course of their experiments is such that, had this effect occurred in the present study, it would have been visible. We do not explain the apparent discrepancy, but we note it.

In contrast, BQ-788 caused strong vasoconstriction whether infused alone or in the presence of \( \text{L-NAME} \), indicating a tonic vasodilator effect of ET-B activation that is substantially independent of nitric oxide. Because nonselective endothelin receptor blockade blunted the constrictor response to \( \text{L-NAME} \), the constrictor response to BQ-788 alone is not explicable in simply additive terms. One possible explanation for this finding arises from the fact that ET-B is a clearance receptor (10) so that BQ-788 can result in elevated circulating endothelin levels and increased occupancy of ET-A receptors. Because drugs were infused directly into the renal artery, or into ~10% of cardiac output, we feel it is unlikely that the concentration of BQ-788 reached blocking levels in the lungs, an important site of endothelin clearance (10). We suggest instead that BQ-788-induced vasoconstriction reflects an ET-A-mediated event that normally is damped by ET-B activation, consistent with the conclusion drawn by Just et al. (19). This event is unlikely to be nitric oxide-dependent because there is no interaction apparent between \( \text{L-NAME} \) and BQ-123, and because the constrictor response to NOS inhibition is not affected by BQ-788. The nature of this pathway is not clear, although it is unlikely to involve prostaglandins or an epoxygenase product (20).

Although agonist-induced vasoconstriction and pressure-dependent autoregulation are related phenomena, the relationship is not necessarily close. Vasopressin, ANG II, and phenylephrine can all cause substantial renal vasoconstriction; however, none of them affects kinetics or dynamics of the myogenic mechanism in vivo (17, 33, 42). In addition, both endothelin and nitric oxide modulate the myogenic mechanism under conditions where vasoconstriction is unlikely to be significant (22, 33). Consequently, a study concerning RBF does not necessarily contain implications about autoregulation and nor does an autoregulation study necessarily contain implications about the regulation of RBF.

Less effective myogenic autoregulation was observed in experiment 2 (ET-A blockade) than occurred in experiment 3 (ET-B blockade). The reasons underlying the difference in the efficiency of myogenic autoregulation in the two experiments are unclear, although we have shown elsewhere that the intra-renal arterial cannula itself can impair RBF dynamics, and this may contribute to the difference (33). Parenthetically, susceptibility to manipulation is a general phenomenon, as discussed in Ref. 26. However, the expected response to \( \text{L-NAME} \) (increased gain slope, increased phase peak, increased fractional compensation) was present in both experiments when \( \text{L-NAME} \) was given alone, although somewhat obscured in experiment 3 by the unusually strong rate-sensitive component of myogenic autoregulation that was apparent during the control period.

Blockade of ET-A receptors by BQ-123 caused, at most, a minor reduction in the effectiveness of myogenic autoregulation and did not interfere in any way with the response to \( \text{L-NAME} \). Thus additive responses most simply explain the combined effects of NOS inhibition and ET-A receptor blockade on the myogenic mechanism. Previous studies have shown that ET-A inhibition, per se, did not affect autoregulation assessed by the pressure ramp experiment (4, 25). Consistent with this finding, in this present study, ET-A inhibition did not affect RBF dynamics. The steady-state experiment used previously showed that the left shift of the lower limit of autoregulation that is induced by \( \text{L-NAME} \) was preserved, although perhaps reduced in amplitude, in the presence of ET-A inhibition (25). The time series analysis used here detected that NOS inhibition augmented the myogenic mechanism both in the absence and in the presence of BQ-123. Thus both experimental designs show pronounced modulation of autoregulation by NOS inhibition but little effect on autoregulation of ET-A inhibition or interaction between the two.

A more complex picture emerges from combined NOS inhibition plus ET-B receptor inhibition by BQ-788. Both NOS inhibition and ET-B inhibition by BQ-788 modulated the myogenic mechanism. In the case of NOS inhibition, this is the expected response (17, 33, 40), while in the case of ET-B inhibition, it can be explained as an inhibition of endothelial...
NOS coupled to ET-B. Blockade of ETB receptors in the presence of l-NAME had no further effect on the myogenic mechanism, most likely because no further modulation was possible. Importantly, however, there was no impairment of the myogenic mechanism in this experimental sequence. In contrast, NOS inhibition after ET-B blockade caused negative modulation of the myogenic mechanism, consistent with the impaired autoregulation seen by Kramp et al. (25). The prima facie explanation of this result would be that removal of ET-B influence exposes an action of ET-A signaling that impairs the myogenic mechanism (and autoregulation generally). However, such an interpretation is not consistent with the results of the experiment in which ET-A receptors were blocked, or with previously published studies (25). We do not have an entirely satisfactory explanation for this rather complex interaction, although several conclusions can be drawn.

In our experiments, as in those of other laboratories (19, 25), it is clear that individual effects of ET-A and ET-B selective antagonists, when added together, are not the same as those of nonselective endothelin antagonists. This is true for effects on vascular tone (19, 25, and present results), for autoregulation (25), and for the myogenic mechanism (present results). In each case, it is also difficult to parse the interaction between endothelin blockade and NOS inhibition. Both for vasoconstriction and for modulation of the myogenic mechanism the interaction between nitric oxide and endothelin is clearly non-additive (i.e., nonlinear) and because it is asymmetrical, cannot be explained simply by convergence on a common signal pathway. Instead, one must postulate that there is some degree of hierarchical organization and that nitric oxide acts downstream to ET-B activation. As discussed by Just et al. (19) in considerable detail, all of these results strongly suggest post-receptor interactions not only between ET-A- and ET-B-mediated events, but also between these and nitric oxide-mediated events.

In summary, the current results are largely consistent with, and provide an explanation for, the finding of Kramp et al. (25) that combined NOS inhibition and ET-B receptor blockade impairs autoregulation. They localize the effect to the myogenic mechanism and show that, like the vasoconstrictor interaction, it is asymmetrical; while prior ET-B receptor blockade alters the autoregulatory response to NOS inhibition, subsequent ET-B receptor blockade does not affect augmentation of myogenic autoregulation by NOS inhibition.

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