Interactions Between Vasoconstrictors and Vasodilators in Regulating Hemodynamics of Distinct Vascular Beds


Abstract—We examined whether interactions between angiotensin II (Ang II), endothelin (ET), nitric oxide (NO), and prostaglandins (PGs) differentially regulate perfusion to distinct vascular beds. For this, we blocked either angiotensin AT1 or ET receptors or both and then sequentially inhibited NO and PG synthesis in anesthetized dogs. Blocking Ang II or ET had similar effects on systemic hemodynamics: Mean arterial pressure fell slightly without altering cardiac output. Blocking both caused a synergistic fall in mean arterial pressure and increased cardiac output. Pulmonary vascular resistance was not altered by blocking Ang II, ET, or both but progressively increased during NO and PG blockade in group 2 (which had unblocked ET receptors), suggesting that endogenous ET exerts pulmonary vasoconstriction that is tempered by NO and PGs. In the kidney, blocking Ang II increased regional blood flow (RBF), glomerular filtration rate (GFR), and fractional excretion of sodium (FENa). In contrast, blocking ET did not alter RBF, and it decreased GFR and FENa. Combined Ang II and ET blockade markedly increased RBF without altering GFR, and FENa was maintained at the levels as when only ET was blocked. Sequentially inhibiting NO and PGs decreased RBF when Ang II or ET were blocked but had little effect when both were blocked. Finally, Ang II or ET blockade did not alter iliac blood flow. Inhibiting NO and PGs decreased iliac blood flow when Ang II or ET but not both were blocked. These results suggest that regional differences in the interactions between endogenous Ang II, ET, NO, and PGs are important determinants in systemic, pulmonary, and regional hemodynamics. (Hypertension. 2003;42[part 2]:831-836.)

Key Words: endothelin ■ angiotensin II ■ nitric oxide ■ prostaglandins ■ hemodynamics ■ natriuresis

The maintenance of mean arterial pressure (MAP) depends on a critical equilibrium between renal perfusion and systemic blood pressure.1 In general, increments of renal perfusion pressure trigger compensatory mechanisms that tend to lower MAP, whereas the opposite occurs when renal perfusion pressure is decreased. To a large extent, these cardiorenal interactions are enacted by the participation of humoral systems such as renin angiotensin, prostaglandins (PGs), nitric oxide (NO) and as revealed in more recent studies by endothelin (ET). Because of this, numerous studies have evaluated the effects of angiotensin II (Ang II), ET, NO donors, and PGs by infusing2–5 or blocking one or more of these vasoactive factors.6–10 However, the relative role that each one of these vasoactive factors plays in regulating regional hemodynamics remains incompletely understood. Therefore, the aim of the present study was to determine the relative role of endogenous Ang II, ET, NO, and PGs (as well as their interactions) in regulating systemic and regional hemodynamics (with particular emphasis on renal function) in normal anesthetized dogs. For this purpose, we blocked the effects of either Ang II with the intravenous administration of L158,810 (a selective AT1 receptor antagonist) or ET by the administration of bosentan (a nonspecific inhibitor of ETA and ETB receptors). The possibility that the inhibitory effects of Ang II and ET were independent or additive was further investigated by comparing the effects produced by the blockade of Ang II or ET with the effects produced by the blockade of both of these substances simultaneously. In addition, the compensatory activity of NO and PG after blocking Ang II, ET, or both were investigated by inhibiting the production with either L-NAME or meclofenamate.

Methods

Surgical Preparation
The Institutional Animal Care and Use Committee at the Mayo Foundation approved all experiments. Twenty-eight mongrel dogs of either sex (16 to 26 kg) were maintained on a standard diet then fasted the evening before the experiment (water ad libitum). On the day of the experiment, the dogs were anesthetized with intravenous pentobarbital (30 mg/kg) and then intubated and ventilated.11 The right femoral artery was cannulated for MAP measurements and collecting blood samples; the right femoral vein was cannulated for infusing anilin, pentobarbital (at 3 mg/min for maintenance of anesthesia), and the different drugs. A Swan-Ganz catheter was inserted to measure cardiac output and pulmonary pressures. Flow probes (Transonic Systems Inc) were placed around the proximal segment of the renal artery and the iliac artery to measure regional perfusion through flank incisions. Last, the left ureter was cannulated...
to obtain urine samples. The dog was then placed in prone decubitus position to mimic the normal position and maintained at 37°C during the experiment. We allowed a 60-minute recovery period before obtaining data. After this equilibration period, urine was collected for 15 minutes and blood samples were drawn for determination of basal values.

**Experimental Design**

Animals were divided into 4 groups. All were subjected to the same experimental protocol, which varied only by the initial inhibitor given. In group 1 (n=7), we sequentially blocked AT1 (with L158810; 0.1 mg/kg bolus, maintained with 0.05 mg/kg per hour), NO (L-NAME, 10 μg/kg per minute), and PGs (meclofenamate, 5 mg/kg bolus). In group 2 (n=7) we blocked ET (bosentan, 3 mg/kg bolus, maintained with 7 mg/kg per hour), then NO and PGs as described above. In group 3 (n=7), both AT1 and ET were blocked (L158810; from 4.2 to 3.4 L/min with bosentan), suggesting that the fall in blood pressure seen during these interventions was due to a fall in SVR (data not shown). However, blocking Ang II and ET receptors simultaneously increased CO from 3.6±0.3 to 4.0±0.3 mL/min. As with blood pressure, sequential inhibition of NO and PGs caused progressive declines in CO during either Ang II or ET receptor blockade. However, when both Ang II and ET were blocked, there was little net effect (CO were very comparable to those seen during the control period). Thus, in the animals in whom AT1 and ET receptors were simultaneously blocked, the major reason for the fall in MAP appears to be due to decreased SVR that is not compensated for by the increments in CO.

**Analytical Methods**

Systemic hemodynamics were measured by standard techniques. Regional blood flow was measured by flow probes. Inulin clearance was used to estimate regional blood flow (GFR). Plasma and urine sodium were measured by photocolorimetry to calculate the fractional excretion of sodium.

**Statistical Analysis**

The results are expressed as mean (SEM) values, with a level of significance of P<0.05 (CI, 95%). ANOVA was performed for each dependent variable, using dog, time, and drug terms as the independent variables. The drug effects were then added to the residuals of this ANOVA model, which created a data set adjusted for dog and time. The difference in values in the periods was studied by a 1-way ANOVA of repeated measurements in the case of normal distribution or Friedman repeated-measurement ANOVA on ranks for nonnormal distribution. A Newman-Keuls test for multiple-means comparison was then used to analyze differences.

**Results**

**Changes in Systemic Hemodynamics and Pulmonary Hemodynamics**

With blocking of the AT1 receptors, there was a small but significant decrease in MAP (from 136±4 to 130±5 mm Hg; P=0.002) (Figure 1). Subsequent inhibition of NO synthesis increased MAP back to the control values (137±4 mm Hg), and the ensuing removal of PGs increased MAP to 147±6 mm Hg (P=0.002 versus control). Blocking ET (group 2) also caused a modest fall in MAP (from 138±3 to 132±4 mm Hg; P<0.005). Inhibiting NO synthesis after ET blockade tended to increase MAP back toward control values (to 134±4 mm Hg), whereas the ensuing inhibition of PGs increased MAP to 142±4 mm Hg. Finally, blocking Ang II and ET receptors simultaneously had a synergistic effect on MAP (MAP fell from 136±3 to 119±3 mm Hg; P=0.001), and inhibiting NO did not reverse this. However, the subsequent inhibition of PGs tended to blunt the fall in MAP induced by ET and Ang II blockade (124±4 mm Hg).

Cardiac output (CO) did not change when Ang II or ET receptors were blocked (from 3.6±0.3 to 3.4±0.2 L/min with L158810; from 4.2±0.3 to 4.1±0.3 L/min with bosentan), suggesting that the fall in blood pressure seen during these
Changes in Iliac Blood Flow

Blocking Ang II AT1 receptors also increased the fractional excretion of sodium (FENa) from 0.6 ± 0.2% to 1.1 ± 0.3% (P < 0.05). Although this tended to further increase after inhibition of NO (to 1.63 ± 0.37%) it did not reach statistical significance and was not altered by the subsequent inhibition of PGs (1.40 ± 0.45%). In contrast, blocking ET receptors with bosentan reduced the FENa (from 1.1 ± 0.5 to 0.5 ± 0.2; P = 0.007). Inhibiting NO did not change these values (0.4 ± 0.1%). Inhibiting PGs caused a further decrease in the FENa (to 0.3 ± 0.1%, P = 0.011 versus the previous period) that was significant but quite small. Finally, the changes in the FENa induced by simultaneous Ang II and ET blockade followed by the subsequent inhibition of NO and PGs mimicked those of when only ET was blocked. That is, blockade of the Ang II and ET receptors together decreased the FENa from 1.4 ± 0.5% to 0.7 ± 0.3% (P = 0.005). Inhibition of NO did not alter the FENa any further (0.8 ± 0.3%), but inhibiting PGs again caused a small decrease (0.6 ± 0.2%; P = 0.005 versus the previous period).

Changes in Renal Hemodynamics and Sodium Excretion

Blocking Ang II increased regional blood flow (RBF) from 178 ± 15 to 212 ± 14 mL/min (P < 0.001) (Figure 2). Subsequent inhibition of NO and then PGs caused RBF to fall to 185 ± 10 mL/min, respectively. In contrast, blocking ET receptors did not alter RBF (from 189 ± 17 to 181 ± 13 mL/min; P = NS); however, subsequent inhibition of NO reduced RBF to 163 ± 12 mL/min (P < 0.001 versus basal). This fall in RBF was further enhanced after the ensuing inhibition of PG synthesis to 149 ± 12 mL/min (P < 0.001 versus basal). Despite the lack of effect of ET alone on RBF, simultaneous blockade of Ang II and ET (group 3) markedly elevated RBF (from 156 ± 13 to 221 ± 10 mL/min; P < 0.001). These changes were predominantly due to changes in renal vascular resistance (RVR); Blocking Ang II or ET receptors produced a 21% (P < 0.01) and 2.5% (P < NS) decrease in RVR, respectively; whereas blocking both decreased RVR by 39% (P < 0.005). The elevated RBF in group 3 was not altered by NO inhibition (223 ± 7 mL/min) but decreased partially when PGs synthesis was inhibited (to 198 ± 8 mL/min; P < 0.001 versus previous and control periods).

Ang II blockade also increased GFR from 36.8 ± 2.4 to 46.4 ± 2.0 mL/min (P = 0.01). This initial rise in GFR was followed by a tendency for it to fall toward baseline after NO inhibition (to 42.0 ± 1.4 mL/min; P = 0.06). Subsequent PG inhibition reduced GFR to 35.3 ± 4.0 mL/min (P < 0.05). These last two values were not different than the basal GFR. In marked contrast to Ang II blockade, blocking ET receptors (group 2) caused GFR to fall from 37.1 ± 3.4 to 29.7 ± 1.6 mL/min (P = 0.001). Subsequent inhibition of NO or PGs synthesis had no further effect on GFR after ET blockade (GFR was 32.2 ± 1.5 and 26.9 ± 3.8 mL/min, respectively). As shown by group 3, simultaneous inhibition of Ang II and ET did not significantly alter GFR despite the marked elevation in RBF (GFR went from 34.3 ± 1.2 to 37.1 ± 2.5 mL/min). Furthermore, the subsequent inhibition of NO and PGs synthesis also had no effect on GFR (37.3 ± 2.4 and 35.4 ± 2.3 mL/min, respectively).

Changes in Renal Hemodynamics and Sodium Excretion

Blocking the Ang II AT1 receptors did not significantly change pulmonary vascular resistance (PVR) (from 4.18 ± 0.44 to 4.37 ± 0.47). However, the ensuing blockade of NO increased PVR, and the subsequent inhibition of PG synthesis further increased, suggesting that pulmonary vasoconstrictor was being unmasked in this group. Blocking ET receptors also had no effect on PVR. However, unlike in group 1, subsequent inhibition of NO and PGs did not increase PVR. Interestingly, simultaneous blockade of Ang II and ET receptors did not alter PVR (in marked contrast to that seen on SVR), and as with ET blockade alone, subsequent inhibition of NO and PGs did not increase PVR, suggesting that ET receptors must be intact for PVR to increase during NO and PG synthesis inhibition.
**Time Control Dogs**

Baseline MAP, CO, pulmonary arterial pressure, IBF, RBF, GFR, and FENa were not different from the other groups and did not change throughout the duration of the experiments (data not shown).

**Discussion**

The present study was performed to answer the following related questions: (1) what is the relative contribution of Ang II (through the AT1 receptor) and ET to the regulation of basal systemic and regional hemodynamics (particularly with regard to renal function), and (2) what roles do NO and PGs play in modulating these effects? These questions were addressed by first blocking either Ang II, ET, or both and then sequentially inhibiting the synthesis of NO and then PGs. With this approach, we attempted to disentangle the contribution of each of these factors to the maintenance of MAP, regional vascular tone, and to renal hemodynamic and natriuretic functions.

**Relative Role of Ang II Through Its AT1 Receptor, ET, NO, and PGs in Regulating Systemic Hemodynamics**

We first found that inhibiting either Ang II or ET does not cause marked alterations in the systemic hemodynamics. Indeed, CO did not change, and MAP fell by only 6 mm Hg when either factor was inhibited. This would at first seem to suggest that neither of these hormones plays a critical role in the maintenance of basal systemic blood pressure. However, this may not necessarily be the case, because when both hormones were simultaneously inhibited, blood pressure dropped markedly. This may suggest that Ang II and ET may compensate for each other to maintain systemic blood pressure. That is, when one of the vasoconstrictors is blocked, the other maintains vascular tone, thus preventing blood pressure from decreasing excessively. However, when both are blocked, the compensatory mechanism is more limited, thus blood pressure falls. Indeed, as mentioned above, we saw that combined Ang II and ET blockade caused a 3-fold greater decrease in blood pressure than when either hormone was blocked alone.

It should be noted that this fall in systemic blood pressure was mostly due to peripheral vasodilation, since CO exhibited an increase by 15%. Part of the peripheral vasodilation appeared to have been caused by the imbalance created between the remaining vasoconstrictors and the vasodilators (NO and PGs). Indeed, inhibiting these vasodilators tended to cause progressive increases in blood pressure (groups 1 and 2). However, it is interesting to note when both vasoconstrictors were blocked, inhibiting NO and PGs had little effect on systemic hemodynamics, suggesting that under the present experimental conditions, the vasconstriction commonly observed after removal of NO or PGs is largely due to the unopposed action of Ang II and ET.

**Relative Role of Ang II Through Its AT1 Receptor, ET, NO, and PGs in Regulating Pulmonary Hemodynamics**

In contrast to the systemic circulation, the pulmonary circulation appeared to be heavily influenced by the interactions between ET, NO, and PGs, with Ang II playing little or no role. This is supported by the finding that blocking NO and PGs in group 1 (where the AT1 receptor was blocked and the ET receptors were left unblocked) caused PVR to increase. However, NO and PG inhibition had no effect on PVR when ET was blocked (either by itself or together with Ang II), suggesting that ET was responsible for the increase in PVR. Interestingly, these responses are consistent with the clinical findings in which administering ET blockers, NO, or PGs can lower pulmonary pressures.

**Relative Role of Ang II Through Its AT1 Receptor, ET, NO, and PGs in Regulating Renal Function**

We found that the AT1 receptor antagonist increased RBF by 21%, whereas MAP fell by only 4%, suggesting that the renal vasculature is exquisitely sensitive to Ang II. This is consistent with the considerable evidence that shows that the renovascular bed is more sensitive than the systemic vasculature to Ang II. On the other hand, blocking the ET receptors (group 2, period 1) caused the same small decline in MAP as Ang II blockade but had an insignificant effect on RBF. A fall in RBF only became apparent after NO was blocked. Thus, ET did not seem to have the same predilection for the renal vasculature as Ang II. This lack of effect of the ET antagonists on basal renal tone is consistent with previous studies in rat kidneys that also suggest that the balance between Ang II and NO/PGs is more important in the maintenance of basal renal vascular tone than ET. However, although ET blockade did not alter RBF significantly when Ang II was present, its effect was striking when Ang II was blocked (group 3); RBF increased dramatically (by 46%). Furthermore, it is tempting to speculate that the lower RBF (by 30%) in the last period of group 1 (where Ang II, NO, and PGs were blocked) compared with the same period of group 3 (where all the factors were blocked) is due to ET-mediated vasoconstriction. Thus, taken together, these observations support the concept that the renal vasoconstrictor effect of endogenous ET is unmasked by inhibiting NO and PGs and that ET may be important in regulating RBF in conditions in which Ang II is low or absent.

Although it is difficult to draw firm conclusions regarding changes in afferent and efferent resistances from whole kidney clearance data, most of the changes induced by Ang II blockade are consistent with changes primarily in afferent arteriolar resistance. For instance, the magnitude of the increase in GFR after Ang II blockade was the same or greater than the increase RBF, suggesting mainly afferent arteriole vasodilation. This response is somewhat surprising because under most circumstances, Ang II acts for the most part on the efferent arterioles; consequently, its removal should not result in such a significant increase in GFR. However, Ang II has also been shown to constrict the afferent arteriole and thus may predominate in the current experimental setting. Nevertheless, we cannot discard the possibility that Ang II blockade–induced increases in the filtration coefficient may also have accounted for the present findings.

In contrast to Ang II blockade, ET blockade produced a rather large decrease in GFR, which is surprising in view of its marginal effect on RBF. This effect was not further altered by
the subsequent blockade of NO and PGs. The decrease in GFR may have been due to a predominant efferent arteriole vasodilation. This explanation is supported by several micropuncture\textsuperscript{22} and in vitro microperfusion\textsuperscript{23} studies that have found that efferent arterioles are more sensitive than afferent arterioles to ET administration. However, we again cannot rule out that changes in the ultrafiltration coefficient accounted for the changes in GFR, although this would be unusual, since most studies show either no change or decreased permeability during ET infusion. Interestingly, simultaneous blockade of Ang II and ET receptors caused a very large increase in RBF without a change in GFR, suggesting that vasodilation was present in both the afferent and efferent arterioles (as would have been expected by the results obtained using the AT\textsubscript{1} or ET blockers alone). Since blockade of either Ang II or ET are expected to increase (or at least not change) the ultrafiltration coefficient, it seems unlikely that decreased glomerular permeability could account for the marked divergence between RBF and GFR. Finally, the fact that the inhibition of NO and PGs did not alter GFR after combined Ang II and ET blockade may further support the concept that the renal vascular actions of NO and PG inhibition are due to the unopposed action of Ang II and ET.

Interestingly, the changes in FENa were independent of the changes in whole kidney hemodynamics. Inhibiting AT\textsubscript{1} receptors markedly increased the FENa in the presence of a modest fall in MAP, suggesting that the increase in FENa can be attributed to the withdrawal of the antinatriuretic effect of Ang II. Indeed, low doses of Ang II are well known to directly increase proximal tubular reabsorption of sodium\textsuperscript{9,24,25} and also stimulate distal reabsorption through stimulation of aldosterone secretion.\textsuperscript{26} However, since AT\textsubscript{1} blockade also increased RBF and GFR, the increased FENa may have also been due to increased renal interstitial pressure subsequent to the elevation in RBF and GFR. An alternative explanation is that the natriuretic effects of ET were left unbalanced after blocking Ang II, thereby increasing the FENa. This explanation is consistent with the fact that inhibiting ET receptors caused a marked fall in the FENa, suggesting that endogenous ET is exerting a natriuretic effect. It is also noteworthy that when both Ang II and ET were blocked, the FENa decreased by the same magnitude as when only ET is blocked. This is somewhat surprising in light of the powerful natriuretic effects of Ang II blockade, suggesting that the natriuretic action of ET may predominate over any other effect that favors sodium excretion. The antinatriuretic effect of ET blockade probably was due to withdrawal of the direct renal effects of ET. Infused ET\textsuperscript{27} has a strong natriuretic effect, which may be due to decreased proximal tubular sodium reabsorption\textsuperscript{28}\textsuperscript{29} or enhanced ET\textsubscript{1} receptor–induced NO and PG synthesis in the kidney.\textsuperscript{5,30,31} Finally, because blocking Ang II or ET may have altered the intrarenal distribution of blood flow, it is possible that changes in the intrarenal hemodynamics may have produced the changes in the FENa. Overall, these findings raise the intriguing possibility that ET is the predominating factor in regulating sodium excretion.

Relative Role of Ang II Through Its AT\textsubscript{1} Receptor, ET, NO, and PGs in Regulating Iliac Hemodynamics

The iliac circulation was not significantly altered when either Ang II or ET was blocked, suggesting that under these conditions, the endogenous Ang II and ET do not play a role in regulating iliac blood flow. However, it should be noted that when either ET or Ang II were left unblocked, inhibiting NO and PGs caused progressive vasoconstriction, whereas when both Ang II and ET were blocked, subsequent inhibition of NO and PGs had no effect. This suggests that the iliac circulation is similar to the systemic circulation in that the pressor effects of NO and PG blockade is largely mediated by endogenous ET and Ang II.

The limitations of the current study are mainly due to the following issues. First, we only studied the blockers in the order described. It is possible that if we would have blocked NO and PGs first, other compensating factors would have been stimulated and thus the subsequent inhibition of Ang II or ET may have yielded different results. Second, these experiments were conducted in anesthetized dogs. Anesthesia itself (or through compensatory mechanisms induced by anesthesia) may alter the responses. Finally, other factors that we could not account for (ie, changes in sympathetic activity or baroreceptor responsiveness, and so forth) may have also influenced the responses. We acknowledge that these issues are potential drawbacks to our study, but it is important to note that all of the available techniques have similar drawbacks (increased stress in conscious restrained animals, and so forth).

In conclusion, the results of this study suggest that specific interactions between Ang II, ET, NO, and PGs occur in the diverse vascular beds. These interactions ultimately regulate regional vascular tone and consequently perfusion to the target tissues. In the acutely anesthetized dog, it appears that the balance between Ang II, NO, and PGs predominantly regulate renal hemodynamics, whereas interactions between ET, NO, and PGs are more important in regulating renal salt excretion and pulmonary hemodynamics.

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References


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