Prevention of the Cardiovascular and Renal Effects of Angiotensin II by Endothelin Blockade

Abderraouf Herizi, Bernard Jover, Nathalie Bouriquet, Albert Mimran

Abstract—Angiotensin II (Ang II) stimulates the release and gene expression of endothelin-1 in isolated vascular smooth muscle cells. In 47 Sprague-Dawley rats, we assessed the influence of concomitant treatment by the mixed ET<sub>A</sub>/ET<sub>B</sub> endothelin receptor antagonist bosentan (30 mg/kg per day, gavage) on the effect of a 10-day infusion of Ang II (200 ng/kg per minute, SC, osmotic pump) on arterial pressure, renal hemodynamics (microsphere method), albuminuria, cardiac weight, and carotid structure. Ang II increased systolic arterial pressure (SAP) by 49±7 mm Hg. Although bosentan alone did not affect SAP, the development of Ang II–induced hypertension was entirely prevented by the endothelin antagonist. In addition, the reduction in renal blood flow induced by Ang II (4.9±0.3 versus 7.4±0.2 mL·min<sup>-1</sup>·g<sup>-1</sup> in control rats) was prevented by concomitant administration of bosentan (8.8±0.8 mL·min<sup>-1</sup>·g<sup>-1</sup>). The marked increase in albuminuria observed in rats infused with Ang II (2524±961 versus 91±6 μg/24 h in control rats) was prevented by bosentan. Similarly, bosentan abolished the increase in heart weight index (from 2.96±0.03 to 3.41±0.08 mg/g body weight) and carotid media thickness (from 73±14 to 108±6 μm) induced by Ang II infusion. Of interest, the dipsogenic action of Ang II was not influenced by bosentan. In conclusion, endogenous endothelin contributes to the cardiovascular and renal effects of Ang II. (Hypertension. 1998;31[part 1]:10-14.)

Key Words: endothelin • angiotensin II • hemodynamics • hypertrophy • albuminuria

In experimental animals, the chronic administration of Ang II results in a dose-dependent rise in arterial pressure, cardiac, and peripheral vascular hypertrophy and proliferation, and injury of small intrarenal arterial vessels (focal fibrinoid necrosis and medial hyperplasia). As a consequence of the increase in arterial pressure and exaggerated glomerular capillary permeability and pressure, an increase in albuminuria and the development of glomerulosclerosis are observed during chronic Ang II infusion. Such a renal effect of Ang II mimics the elevated albuminuria often seen in renin-dependent forms of human hypertension such as that associated with renal artery stenosis and thrombosis. Nevertheless, Ang II may have blood pressure–independent effects on target organs, as suggested by the development of cardiac hypertrophy in rats infused with a nonpressor dose of Ang II or when the increase in arterial pressure was prevented by hydralazine.

In recent years, it was reported that Ang II is a powerful stimulator of ET-1 release by cultured vascular smooth muscle and endothelial cells. In addition, Ang II stimulated the expression of preproendothelin-1 mRNA and the ET-1 gene in cultured rat and bovine endothelial cells, rat vascular smooth muscle cells, cardiomyocytes, and renal mesangial cells. In fact, it was demonstrated that part of the hypertrophic and mitogenic effects of Ang II may be mediated by ET-1 as suggested by the influence of monoclonal antibodies to ET-1, inhibition of endothelin–converting enzyme by phosphoramidon, and blockade of type A endothelin receptors by BQ-123. Vascular ET-1 may also act as an amplifier of the vasoconstrictor effect of Ang II, because in normotensive rats a dose of ET-1 devoid of pressor effect potentiated the effect of a nonpressor dose of Ang II, thus resulting in an increase in arterial pressure.

In the present studies, the influence of the mixed ET<sub>A</sub> and ET<sub>B</sub> receptor antagonist bosentan on the development and maintenance of hypertension as well as organ damage (kidney, heart, and large vessels) associated with chronic infusion of Ang II was assessed in rats.

Methods

Experiments were carried out in 47 male Sprague-Dawley rats (Iffa-Credo, L’Arbresle, France) weighing 275 to 300 g and maintained on a normal sodium intake (sodium-free rat chow containing <5 mmol sodium per kg and distilled water containing 77 mmol sodium per liter as drinking fluid). Animals were placed in individual metabolic cages at least 1 week before studies and randomly assigned to the four experimental groups.

After a 3-day baseline period, Ang II or its vehicle (distilled water) was infused alone (Ang II and control groups, respectively, consisting of 13 rats each) or in association with the oral administration of bosentan (Ang II–Bos and Bos groups consisting of 13 and 8 rats, respectively). Ang II (Sigma Chemical Co) was infused subcutaneously via osmotic pumps (model 2002, Alza Corp) at a dose of 200 ng·kg<sup>-1</sup>·min<sup>-1</sup> for 10 days and bosentan (Ro 47–0203, donated by Dr. Jean-Paul Clozel and Dr. Martine Clozel of F Hoffmann–La Roche Ltd, Basel, Switzerland) was administered once daily (between 8 and
At the end of experiments, eight rats from each group were
killed by an intraventricular injection of pentobarbital sodium,
and the heart and kidneys were removed and weighed for radioactivity
counting and calculation of heart and kidney to body weight ratio,
respectively.

In the remaining five rats from the Ang II, Ang II–Bos, and
untreated groups, carotid media thickness and lumen diameter were
estimated. Briefly, rats were anesthetized with pentobarbital (60
mg/kg, IP), and the right carotid was catheterized (PE 50, Merck-Clevenot) and washed
into the lower aorta via the left femoral artery. Both catheters were tunneled subcutaneously and exteriorized
at the back of the neck. After a 3-hour recovery period, catheters were connected to a pressure transducer (Statham P23ID), and arterial pressure and heart rate were continuously recorded for 30 minutes in conscious, freely moving animals. During the intraventricular injection of microspheres, blood was sampled at a rate of 0.5 mL/min for 2 minutes for radioactivity counting and determination of plasma concentrations of sodium, potassium, and creatinine. Animals were then killed by an intraventricular injection of pentobarbital sodium, and the heart and kidneys were removed and weighed for radioactivity counting and calculation of heart and kidney to body weight ratio, respectively.

In all samples, concentrations of sodium and potassium were measured by flame photometry and creatinine concentration by a colorimetric method. Urinary excretion of albumin was determined by immuno-nephelometric method.15

Results were expressed as mean±SEM and analyzed by one- or
two-factor ANOVA for repeated measures as appropriate. Differences
between groups were assessed by the Fisher’s protected least significant
difference test. Within-group differences were evaluated by the
Student’s t test for paired values. A value of P<.05 was considered
statistically significant.

Results

Arterial Pressure
As depicted in Fig 1, SAP measured by the tail-cuff method in
conscious rats remained stable throughout studies in the
control untreated group (basal 127±1 mm Hg and final value
125±1 mm Hg) and rats treated by bosentan alone (basal
125±4 mm Hg and final value 128±3 mm Hg). In rats infused
with Ang II alone, SAP rose progressively from a basal value of
124±2 mm Hg to 139±4 on day 1, 148±5 on day 3, and
173±6 mm Hg on the final day. Administration of bosentan,
24 hours before and for the duration of studies, strikingly
attenuated the development of Ang II–induced hypertension.
Although the final SAP level (136±2 mm Hg) was similar to
baseline (130±3 mm Hg), SAP increased by 14±4 mm Hg on
day 3 (P<.02), a value significantly higher than control
untreated rats (P<.02) and similar to that observed in rats
infused with Ang II alone (24±5 mm Hg).

Metabolic Parameters
Within the 10-day period of the study, body weight gain was
significantly attenuated in the Ang II group (27±6 g) when
compared with untreated (43±4 g) and bosentan-treated
(43±7 g) groups. Concomitant administration of bosentan
prevented the body growth impairment associated with
chronic Ang II (43±5 g).

As shown in Fig 2, water intake markedly increased during
Ang II administration. The dipsogenic effect of Ang II was not
affected by bosentan, which otherwise had no influence on
water intake when given alone in normotensive rats. At the
end of studies, plasma concentration of sodium and potassium
and hematocrit level were similar in all groups.

Renal Hemodynamics and Function
After recovery from ether anesthesia, intra-arterial MAP was
111±1 and 134±3 mm Hg in untreated control rats and Ang
II–infused rats. Although MAP in bosentan–treated normoten-
sive rats was similar to that in control rats (116±7 mm Hg),
bosentan entirely prevented Ang II–induced hypertension
(106±6 mm Hg). As depicted in Fig 3, the reduction of
cardiac output and renal blood flow, as well as the increase in
total peripheral and renal resistances, associated with chronic
administration of Ang II, were totally prevented by concomi-
tant administration of bosentan. Treatment of normotensive
rats with bosentan had no detectable effect on systemic and
renal hemodynamics.

Final serum creatinine was similar in all groups. Creatinine
clearance calculated from 24-hour urine and serum creatinine
values was higher in Ang II–infused rats when compared with
control animals (497±27 versus 343±21 µL/min per g kidney

Selected Abbreviations and Acronyms
Ang II = angiotensin II
ET-1 = endothelin-1
MAP = mean arterial pressure
SAP = systolic arterial pressure

Analytical Methods and Statistical Analysis
In all samples, concentrations of sodium and potassium were measured by flame photometry and creatinine concentration by a colorimetric method. Urinary excretion of albumin was determined by immuno-nephelometric method.15

As depicted in Fig 1, SAP measured by the tail-cuff method in
conscious rats remained stable throughout studies in the
control untreated group (basal 127±1 mm Hg and final value
125±1 mm Hg) and rats treated by bosentan alone (basal
125±4 mm Hg and final value 128±3 mm Hg). In rats infused
Interestingly, creatinine clearance was still higher in the group treated with Ang II and bosentan than in control animals (486 ± 58 µL/min per g kidney wt). As shown in Fig 4, urinary excretion of albumin markedly increased in Ang II–infused rats (from 217 ± 47 to 2524 ± 961 µg/24 hours, P < .01), whereas it remained constant in untreated and bosentan–treated control rats. Concomitant bosentan administration abolished the proteinuric effect of Ang II.

Cardiovascular Changes

As summarized in the Table, a significant increase in heart weight index was observed in Ang II–treated rats (3.41 ± 0.08 versus 2.96 ± 0.03 mg/g body wt in untreated animals, P < .05), and bosentan prevented the cardiac hypertrophic effect of Ang II. In addition, chronic treatment with Ang II was associated with a consistent incremental increase in the carotid media thickness without a change in the lumen diameter. The mean value of carotid media thickness was restored to control values in rats treated by Ang II and bosentan.

Discussion

In the present studies, the development of hypertension, as well as cardiac and vascular hypertrophy, resulting from chronic infusion of angiotensin II was prevented by the concomitant administration of the mixed endothelin receptor antagonist bosentan. Moreover, renal vasoconstriction and proteinuria associated with Ang II were similarly abolished by bosentan. Interestingly, the dipsogenic effect of Ang II was not affected by bosentan.

In previous studies conducted in renin–dependent forms of experimental hypertension, such as that observed in the early phase of partial unilateral renal ablation (ligation of two of the three branches of the left renal artery), acute administration of the endothelin receptor A antagonist BQ-123 was associated with a fall in arterial pressure similar to that induced by captopril.17 In rats with renal artery clipping and intact contralateral kidney, bosentan prevented approximately 40% of the early increase in arterial pressure.18 However, no effect of acute blockade of ET A receptors by FR139317 on arterial pressure was found in two-kidney, one clip hypertensive rats studied 4 weeks after clipping;19 and administration of bosentan 8 weeks after clipping (at a phase characterized by cardiac and vascular overexpression of the ET-1 gene) had no effect on arterial pressure or the structure of small vessels.20,21 These observations suggest a major contribution of endothelin to the increase in arterial pressure, only during the early (and probably highly...
reduced by the release of nitric oxide and prostacyclin that results from the lack of blockade of type B endothelin receptors\(^23\) may have contributed to the effect of LU135252. Our findings clearly suggest that endothelin markedly influenced the hypertensive effect of Ang II, at least when administered at a dose of 200 ng · kg\(^{-1}\) · min\(^{-1}\), which was shown to result in a threefold increase in the circulating concentration of the octapeptide.\(^2,24\)

The present results, obtained in chronically treated rats, extend previous studies showing that bosentan shifted to the right the dose-response curve of arterial pressure and cardiac output to acute Ang II.\(^5,5\) The observed protective effect of endothelin inhibition is in favor of an important role for the stimulation of endothelin release by Ang II, probably via activation of type 1 Ang II receptors.\(^7,26\)

Whether the influence of endothelin is specific to Ang II remains to be established. It was recently reported that chronic norepinephrine infusion augmented the ventricular expression of ET-1 mRNA and that bosentan prevented the development of cardiac hypertrophy; unfortunately no measurement of arterial pressure was reported.\(^27\) In addition, Emori et al.\(^28\) observed that Ang II and vasopressin both stimulated the release of ET-1 and the expression of preproendothelin-1 mRNA by cultured bovine endothelial cells.\(^5\)

In the present studies, bosentan prevented cardiac hypertrophy as well as carotid artery structural changes associated with Ang II infusion, probably as a consequence of the lack of increase of arterial pressure. This is in agreement with previous studies that suggested that endothelin blockade affects hypertension-associated vascular remodeling only when a significant antihypertensive effect is observed.\(^21,29,30\) Moreover, bosentan abolished the renal vasoconstrictor effect and the increase in albuminuria associated with chronic Ang II infusion. Among mechanisms of the Ang II–induced increase in albuminuria are the increase in systemic pressure, a rise in intraglomerular capillary pressure resulting from preferential constriction of the efferent glomerular arteriole, and an increase in the glomerular permeability to albumin and possibly other macromolecules.\(^4\)

Prevention of the albuminuric effect of Ang II by bosentan could be the consequence of the lack of increase in systemic pressure and blockade of the renal vasoconstrictor effect of exogenous Ang II, at least within the rather short period of administration of the octapeptide.

### Influence of Bosentan on Cardiovascular Changes Induced by Chronic Ang II Infusion

| Group                      | Untreated | Bosentan | Ang II | Ang II–Bosentan |
|----------------------------|-----------|----------|--------|----------------|-----------------|
| Initial tail-cuff pressure, mm Hg | 127±1     | 125±4    | 124±2  | 130±3          |
| Final tail-cuff pressure, mm Hg | 125±1     | 128±3    | 173±6* | 136±2†         |
| Body weight gain, g/10 d  | 43±4      | 43±7     | 27±6*  | 43±5†          |
| Heart weight index, mg/g body wt | 2.96±0.03 | 2.98±0.15 | 3.41±0.08* | 3.01±0.07† |
| Kidney weight index, mg/g body wt | 7.55±0.20 | 7.98±0.50 | 8.25±0.15 | 7.79±0.25 |
| Carotid cross-sectional area, mm² | 0.10±0.01 | ND       | 0.14±0.01* | 0.12±0.01† |
| Carotid media thickness, μm | 77±4      | ND       | 108±6* | 73±14†         |
| Carotid lumen diameter, μm | 767±6     | ND       | 781±37 | 831±24         |
| Carotid media/lumen       | 0.101±0.005 | ND   | 0.140±0.012* | 0.090±0.019† |

ND indicates not determined. Values are mean±SEM.

\(^*P<.05\) vs untreated rats, \(^†P<.05\) vs Ang II alone.
As shown in Fig 2, no influence of the endothelin antagonist on the known dipsogenic effect of Ang II was observed. This suggests that either bosentan did not cross the blood-brain barrier and thus reach endothelin receptors located within the central nervous system or that endothelin does not contribute to the dipsogenic effect of Ang II. In fact, it was reported that intracerebroventricular injection of endothelin exerted an antidipsogenic effect through type A receptors and that central administration of the type A–receptor antagonist BQ-123 significantly accentuated the drinking response elicited by Ang II.

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