Natriuretic peptide gene expression in DOCA-salt hypertension after blockade of type B endothelin receptor

LILIANA G. BIANCIOTTI AND ADOLFO J. DE BOLD
University of Ottawa Heart Institute and the Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, Ontario K1Y 4W7, Canada

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Biancotti, Liliana G., and Adolfo J. de Bold. Natriuretic peptide gene expression in DOCA-salt hypertension after blockade of type B endothelin receptor. Am J Physiol Heart Circ Physiol 282: H1127–H1134, 2002; 10.1152/ajpheart.00468.2001.—We investigated the effect of long-term in vivo blockade of the ET-1 receptor subtype B (ETB) with A-192621, a selective ETB antagonist, on atrial and ventricular natriuretic peptide (NP) gene expression in deoxycorticosterone acetate (DOCA)-salt hypertension. In this model, stimulation of the cardiac natriuretic peptide (NP) and the endothelin system and suppression of the renin-angiotensin system is observed. DOCA-salt induced significant hypertension, cardiac hypertrophy and increased NP plasma and left atrial and right and left ventricular NP gene expression. ETB blockade per se produced hypertension and left ventricular hypertrophy but induced little change on the levels of ventricular NP and only increased left atrial natriuretic factor (ANF) mRNA levels. Combined ETB blockade/DOCA-salt treatment worsened hypertension, increased left ventricular hypertrophy and induced right ventricular hypertrophy. All animals so treated had increased ventricular NP gene expression. Collagen III and β-myosin heavy chain gene expression were enhanced in both the right and the left ventricle of DOCA-salt hypertensive rats. The results of this study suggest that the ETB receptor does not participate directly in the modulation of atrial or ventricular NP gene expression and that this receptor mediates a protective cardiovascular function. ETB blockade can induce significant ventricular hypertrophy without an increase in ANF or brain NP gene expression.

deoxycorticosterone acetate

THE LEVEL OF EXPRESSION of the cardiac natriuretic peptides (NP), atrial natriuretic factor (ANF), and brain natriuretic peptide (BNP), as well as circulating plasma levels, is increased by hemodynamic overload through mechanisms that are believed to involve both mechanical and neuroendocrine stimuli (7). The activation of the cardiac NP system serves to counterbalance the activity of systems that tend to increase extracellular fluid volume and blood pressure such as the renin-angiotensin-aldosterone and sympathetic systems (6). Although much information has been gathered regarding the regulation of NP gene expression and release through in vitro approaches, comparatively little is known regarding the long-term regulation of NP in the heart. Using a model of renovascular hypertension in the rat, we have previously found that there are two components that contribute to chronic increased NP gene expression. One component is independent of load and is concurrent with the hypertrophic process of chronically overloaded hearts whereas the other is dependent on hemodynamic load (26). We also reported that the expression of ANF and BNP in the ventricles and atria is differentially regulated. That is, in the atria, NP expression appears to be governed by mechanical stimuli (atrial muscle stretch) whereas in the ventricles the expression of ANF and BNP seems to be mainly dependent on the endocrine environment because the ventricular expression of NP could be downregulated by treatment with a low dose of an angiotensin-converting enzyme (ACE) inhibitor in hypertensive animals whereas atrial NP production did not change with either a low or a high dose of the ACE inhibitor (26).

More recently, we (1, 2) reported investigations that lend further support to the concept that transcriptional control of NP production is not the same in atria as in ventricles. We observed in deoxycorticosterone acetate (DOCA)-salt hypertension as well as in renovascular hypertension, that chronic selective blockade of the endothelin-1 (ET-1) receptor subtype A (ETA) reduces ventricular ANF and BNP gene expression and cardiac hypertrophy. Furthermore, blood pressure was normalized after ETA blockade in DOCA-salt hypertensive rats and partially prevented in renovascular hypertensive rats. However, in the atria, NP stores or NP steady-state mRNA levels were not modified. These investigations showed that whereas modulation of ventricular NP production by hormones in vivo resembles that which is obtained in vitro using cultures of neonatal cardiocytes, modulation of atrial NP production in vivo is largely independent of the endocrine factors so far tested. Atrial NP production, however, appears mainly determined by atrial wall stretch as modified by changes in blood volume (38) although in vitro...
preparations such as the isolated atrial preparation (27), it is possible to modify atrial NP production with agents such as endothelin albeit at fairly high concentrations.

ET_A and ET_B are expressed in the atrial and ventricular myocardium as well as in the atrioventricular conducting system and endocardial cells (24). The role of the ET_A receptor in NP gene expression has been fully addressed in physiological and pathophysiological situations. ET-1 acutely stimulates ANF and BNP mRNA synthesis and NP secretion from cardiocytes in culture signaling through the ET_A receptor (3, 34, 36). Whether the ET_B receptor plays a role in NP chronic enhancement induced by pressure and volume overload remains unknown. ET_B receptor activation induces endothelium-dependent relaxation through the release of nitric oxide and prostacyclin (33). There is also significant evidence that the hypertension after ET_B blockade is the result of decreased renal sodium clearance (12, 28). Furthermore, ET_B is also considered a clearance receptor for circulating endothelins (8, 11, 32).

The aim of the present work was to define the possible role of the ET_B receptor subtype on cardiac NP gene expression. To this purpose, we investigated the effect of ET_B blockade with A-192621, an orally active selective ET_B antagonist, on atrial and ventricular NP gene expression and production in DOCA-salt hyper- tension. In this model of hypertension, the cardiac NP gene expression and production in DOCA-salt hypertrophy, including myosin heavy chain (MHC) isoforms mRNA steady-state levels.

METHODS

Male Sprague-Dawley rats weighing between 125 and 150 g were fed ad libitum and housed under conditions of constant temperature and humidity, with a 12-h light-dark cycle. All experiments were performed according to the recommendations of the Canadian Council of Animal Care.

DOCA-salt hypertension was induced by weekly subcutaneous injections of 30 mg/kg DOCA (Sigma) in sesame seed oil as vehicle and the administration of 1% saline in the drinking water for 5 wk. A group of rats also received 30 mg·kg⁻¹·day⁻¹ of A-192621 or vehicle twice daily by gavage. The oral administration of A-192621 in a dose of 30 mg·kg⁻¹·day⁻¹ has been shown to inhibit ET_B receptor-mediated depressor and pressor responses (29). After 5 wk, blood pressure was measured in conscious rats by tail plethysmography (Narco Biosystem; Austin, TX) and the average of three pressure readings was recorded. The animals were euthanized by decapitation, and trunk blood samples were collected into ice-cold tubes containing 0.1 ml of 15% K3-EDTA. Blood samples were centrifuged at 2,000 g for 30 min at 4°C, and plasma was kept at −80°C until assayed for immunoreactive ANF and BNP. The hearts were rapidly excised, and the four chambers were dissected, weighed, and quickly frozen in liquid nitrogen and kept at −80°C. The intratriatal and interventricular septa were included with the respective left chamber.

Total mRNA extraction and Northern blot analysis. Total mRNA extraction and Northern blot analysis were performed as previously described (26). Briefly, total RNA was extracted and electrophoretically separated in agarose formaldehyde gel, followed by blotting to nylon membranes (Hybond N+, Amersham). Membranes were hybridized with cDNA and oligonucleotide probes as detailed in previous studies. The following cDNA probes were used: 1) a 900-bp EcoRI/HindIII fragment containing the full length rat ANF cDNA, 2) a 595-bp SalI fragment containing the full-length BNP cDNA, 3) a 5-bp EcoRI/SalI fragment of the mouse 28S rRNA probe, 4) a 2-κb BamH VBglII fragment of the mouse phosphoryl-erase kinase gene cDNA, and 5) rat 1α-collagen III cDNA containing 1,300 bp of the 3′ noncoding and coding regions. The two oligonucleotides used were 39 and 24 base fragments specific for the unique regions in the 3′ untranslated regions of the rat α-MHC and β-MHC genes.

The cDNAs were labeled with 5′-α32P]dCTP (3,000 Ci/ mmol) using the Megaprime DNA labeling system. The oligonucleotides were labeled with γ-32P]ATP (3,000 Ci/mmol) using a 5′ end-labeling kit. All were purchased from Amersham. Before additional probing, bound radioactivity was stripped off the membranes by washing with 10 mM sodium citrate (pH 6.8) and 0.25% sodium dodecyl sulfate at 100°C for 10 min. Autoradiographs were scanned using a laser densitometer and the scanning values for ANF, BNP, colla- gen III, and MHC isoforms mRNAs were normalized to 28S rRNA or phosphoglycerate kinase mRNA to correct for differences in the amount of RNA applied and for transfer efficiency.

Plasma extraction and radioimmunoanalysis for NP. NP were extracted from plasma and assayed as previously described (1). Anti-rat ANF (99–126) and anti-BNP (64–95) sera were purchased from Peninsula Laboratories (Belmont, CA) and showed less than 0.01% cross-reactivity with BNP and ANF respectively.

Analysis of results. Data are expressed as means ± SE. Statistical analysis was performed by one-way analysis of variance, followed by a post hoc Bonferroni test using Systat. A P value of ≤0.05 was considered statistically significant.

RESULTS

Systolic blood pressure was significantly elevated in DOCA-salt-treated rats as well as in the rats receiving the ET_B blocker alone. Blood pressure was further increased in DOCA-salt rats after ET_B blockade. The relative left ventricular weight (left ventricle weight-to-body weight ratio) was increased in DOCA-salt hypertensive rats and those receiving the ET_B blocker. ET_B blockade aggravated left ventricular anatomical hypertrophy in the DOCA-salt group. The relative right ventricular weight was not modified in DOCA-salt-treated rats or those receiving A-192621 alone. However, ET_B blockade significantly increased relative right ventricular weight in DOCA-salt-treated rats (Table 1).

ANF and BNP plasma level changes closely paralleled each other through the different treatments (Fig. 1). DOCA-salt treatment significantly increased plasma levels of both hormones. ET_B blockade did not modify plasma levels in DOCA-salt-treated rats. By itself, the blocker increased ANF but not BNP plasma concentra-
Ventricular and left atrial ANF gene expression was enhanced in DOCA-salt-treated rats. ETB receptor blockade increased left atrial ANF transcript levels in control and DOCA-salt-treated rats. Ventricular ANF gene expression was not affected in rats treated with the ETB blocker (Fig. 2). BNP gene expression followed a pattern similar to that of ANF except that the level of expression of BNP was significantly elevated not just in the left atrium as for ANF but also for the right atrium (Fig. 3).

DOCA-salt-treated rats showed increased β-MHC gene expression in both ventricles and a decrease in the α isoform only in the left ventricle. The administration of the ETA antagonist did not modify α-MHC transcript levels but further increased the β isoform expression in both ventricles (Fig. 4 and 5).

Collagen III gene expression was enhanced in both right and left ventricles of DOCA-salt hypertensive rats but ETB blockade only reduced collagen transcript levels in the right ventricle of DOCA-salt-treated rats (Fig. 6).

DISCUSSION

Chronic hemodynamic overload enhances NP gene expression leading to an increase in its circulating levels. This increased expression is deemed a counterbalancing mechanism after activation of pressure- and extracellular volume-promoting systems such as the renin-angiotensin-aldosterone and sympathetic systems. In vitro systems such as cardiocyte cultures and whole tissue perfusion have helped identify both mechanical and neuroendocrine stimuli that enhance NP gene expression and secretion (3, 7). Much less defined is the role of these stimuli in vivo. There exists accumulating evidence indicating that stimuli thus far identified in vitro may not necessarily play a role in vivo. In addition, there are clear indications that the transcriptional control of NP gene expression in atria, which is the normal site for expression of the endocrine function of the heart, differs from that in ventricles. In experimental DOCA-salt and in renovascular hypertension, for example, blockade of the ETA receptor results in significant reduction in the development of left ventricular hypertrophy and ventricular ANF and BNP gene expression but does not affect atrial gene expression for either of these peptides (1, 2). Similarly, treatment of hypertensive aortic-banded rats with the ACE inhibitor ramipril modifies the ventricular level of expression and degree of left ventricular hypertrophy but has no discernible effect on atrial NP production (26).

Through in vitro investigations, ET-1 has emerged as a potent regulator of ANF release and gene expression mediated by the ETA receptor subtype (4, 34, 35) although in vivo, this applies to ventricular ANF gene expression and not atrial expression. The possible contribution of the ETB receptor in the regulation of stimulated ANF gene expression has not been so far investigated. In the present work we sought to establish the contribution of the ETB receptor subtype signaling to atrial and ventricular ANF gene expression in DOCA-salt hypertension through the use of the orally active selective ETB antagonist A-192621.

DOCA-salt hypertension is a low renin model associated with upregulation of the endothelin system and NP in both atria and, in association with the hypertrophic response, also in the ventricles. Blockade of the ETB receptor resulted in further increase in blood pressure in hypertensive animals. This finding is in line with previous findings (5, 15, 23, 30) and supports a protective role for ET-1 signaling through the ETB receptor in this model of hypertension. The pressor effect of A-192621 may occur as a result of the inhibition of nitric oxide release mediated by the ETB receptor. This assumption is based on previous findings using the same experimental model in which the pressor effect of another selective ETB antagonist (Ro46-8443) was abolished by pretreatment with Nω-nitro-L-arginine methyl ester (5).

The activation of the ETA receptors by increased ET-1 could also contribute to the elevation of blood pressure after the administration of a selective ETB antagonist although the pressor effect of the selective ETB antagonist Ro 46-8443 was reported to be unaffected by the administration of a selective ETA.
antagonist (5). A more recent study however, showed that the administration of ABT-627 (ETα selective antagonist) decreased mean arterial pressure in rats in a high-salt diet treated with the ETβ antagonist A-192621 (31).

ETβ also functions as a clearance receptor for circulating ET-1 mainly in the lungs and kidney (11). Therefore, the elevation of blood pressure after A-192621 administration may be also a consequence of reduced clearance of circulating ET-1. ETβ receptors are upregulated in the renal medulla of DOCA-salt hypertensive rats. They appear to play a protective role by lowering arterial pressure and by promoting salt and water excretion. Renal ETβ receptors located on collecting duct cells can inhibit tubular reabsorption resulting in blood pressure lowering through changes in extracellular fluid balance (9, 13, 31). ETβ also appears to regulate blood pressure in the normotensive state (28). From these findings, it may be speculated that in our work the blockade of renal ETβ receptor may also have contributed to the aggravation of hypertension due to a decreased sodium clearance.

The participation of ET-1 signaling through the ETA receptor in long-term enhanced NP gene expression has been previously studied by us (1, 2). From these studies it is clear that both ANF and BNP are conjoinedly upregulated. ETA receptor blockade reduced ANF and BNP gene expression, systolic blood pressure

Fig. 2. Relative abundance of ANF transcripts in the cardiac chambers after chronic endothelin type B (ETβ) receptor blockade (30 mg·kg⁻¹·day⁻¹ of A-192126) in DOCA-salt hypertensive rats. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control; §§P < 0.05, §§§P < 0.01 and §§§§P < 0.001 vs. DOCA-salt.

Fig. 3. Relative abundance of BNP transcripts in the cardiac chambers after chronic ETβ receptor blockade (30 mg·kg⁻¹·day⁻¹ of A-192126) in deoxycorticosterone acetate (DOCA-salt) hypertensive rats. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control; §§P < 0.05, §§§P < 0.01, and §§§§P < 0.001 vs. DOCA-salt.
as well as left ventricular hypertrophy in DOCA-salt hypertension as well as in renovascular hypertension. It would be expected that the increase in blood pressure induced by ETB blockade found in the present work might result in further enhancement in NP gene expression over that observed with DOCA-salt treatment alone. This was not the case: ETB blockade did not increase ventricular NP transcript levels over that found in animals treated with DOCA-salt alone. These results support the hypothesis that the mechanical stimuli per se is not the only determinant of ventricular NP gene expression and further that the magnitude of the increase in blood pressure does not correlate with the magnitude of the enhancement of NP expression in the ventricles.

Neuroendocrine factors such as the endothelins, angiotensin II and cytokines as well as mechanical stimuli such as volume and pressure overloads stimulate different intracellular pathways that converge in evoking the expression of immediate early genes, the promotion of growth of cardiac cells and the re-expression of fetal genes (10, 16, 18, 22, 25, 37). Both ET<sub>A</sub> and ET<sub>B</sub> endothelin receptor subtypes are expressed in cardiac tissue (17, 24). The ET<sub>A</sub> receptor is expressed to a greater degree than ET<sub>B</sub> in atrial and ventricular cardiocytes but both have been implicated in the pathogenesis of myocardial hypertrophy and are upregulated in the hypertrophied myocardium (25). In the present study, ET<sub>B</sub> receptor blockade enhanced left ventricular hypertrophy in DOCA-salt-treated rats. Moreover it induced right ventricular hypertrophy in the same group. These results suggest that the ET-1 through the ET<sub>B</sub> receptor may exert a negative modulation on the hypertrophic process. Therefore, ET-1 seems to play two opposing roles in the hypertrophic process, one stimulatory effect mediated by the ET<sub>A</sub> receptor and another inhibitory effect mediated by the ET<sub>B</sub> receptor subtype. It is worth noting that blockade of the ET<sub>B</sub> receptor can affect pulmonary hemodynamics (19, 20) and this may possibly contribute to the increased burden imposed to the right ventricle as the result of blood pressure elevation.

Ventricular hypertrophy is characterized by the re-expression of the cardiac fetal gene program, which includes modifications in MHC content and relative isoform content. In addition, the collagen content of the myocardium increases due to the activation of interstitial fibroblasts. In the present study, we determined steady-state mRNA levels for collagen III and MHC isoforms to place within context the changes observed

![Graphical representation of ventricular hypertrophy](image_url)

**Fig. 4.** Relative abundance of α-MHC transcripts in the left and right ventricles after chronic ET<sub>B</sub> receptor blockade (30 mg·kg<sup>-1</sup>·day<sup>-1</sup> of A-192126) in DOCA-salt hypertensive rats. *P < 0.05 vs. control.

**Fig. 5.** Relative abundance of β-myosin heavy chain (MHC) transcripts in the left and right ventricles after chronic ET<sub>B</sub> receptor blockade (30 mg·kg<sup>-1</sup>·day<sup>-1</sup> of A-192126) in DOCA-salt hypertensive rats. **P < 0.01 and ***P < 0.001 vs. control; §P < 0.05 and §§P < 0.01 vs. DOCA-salt.
that ET\textsubscript{B} blockade reduced collagen III gene expression in the right ventricle although not to control levels suggesting that factors other than ET-1 participate in the cardiac remodeling process. In the left ventricle of DOCA-salt-treated rats collagen III gene expression was unaltered after A-192621 administration. The reasons for the discrepancy in the level of collagen III transcripts observed in right and left ventricles after ET\textsubscript{B} blockade is not apparent from this work, but it may be related to the higher hemodynamic burden on the left ventricle.

In the present work, circulating NP levels were increased in DOCA-salt-treated rats reflecting changes in left atrial and ventricular NP changes in gene expression. These findings are the same as those obtained by us in previous investigations of the DOCA-salt model of hypertension (1, 2, 38). A marginally significant increase in ANF plasma levels was observed in animals treated with the ET\textsubscript{B}, whereas BNP plasma levels remained unchanged. This finding is likely due to the fact that the smaller relative increase of BNP released into the circulation after mild stimulation of the endocrine heart is often masked by dilution into the plasma pool (7).

Our results support the view that ET\textsubscript{B}-mediated signaling is a protective factor in the pathogenesis of DOCA-salt hypertension. Previous findings using the spotting lethal rat, which carries a naturally occurring deletion in the ET\textsubscript{B} receptor gene, showed enhanced blood pressure in DOCA-salt hypertensive animals (23). The protective role of the ET\textsubscript{B} receptor also has been suggested in the renal medulla where their density is upregulated in hypertension and may serve to maintain a lower arterial pressure by promoting salt and water excretion (30).

In conclusion, the present findings show that the ET\textsubscript{B} receptor does not participate in the modulation of ventricular NP release and gene expression in the chronic hemodynamic overload that occurs in DOCA-salt hypertension. However, the effect of ET-1 on ventricular NP gene expression in DOCA-salt hypertensive animals appears to be exclusively mediated by the ETA receptor as shown previously (1). The increase in NP gene expression and circulating levels after ET\textsubscript{B} blockade might suggest a role for ET\textsubscript{B} in modulating NP gene expression. However, we have previously demonstrated a partial role for hemodynamic load in determining the level of expression of NP (26), and hence, the changes observed more likely arise from hemodynamic changes (e.g., increased blood pressure) because ET\textsubscript{B} blockade did not further elevate NP plasma levels and cardiac gene expression in DOCA-salt-treated rats after ET\textsubscript{B} blockade. From this and previous work, neither ET\textsubscript{B} nor ET\textsubscript{A} long term blockade affect atrial NP gene expression, further suggesting a nonendocrine regulation of atrial ANF gene expression. ET\textsubscript{B} receptor blockade aggravated hypertension and ventricular hypertrophy. Thus the ET\textsubscript{B} receptor appears to mediate a protective role on the heart and blood pressure regulation in DOCA-salt hypertension.

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**Fig. 6. Relative abundance of collagen III (Coll III) transcripts in the left and right ventricles after chronic ET\textsubscript{B} receptor blockade (30 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} of A-192126) in DOCA-salt hypertensive rats. **\textsuperscript{p} < 0.01 vs. control; §§\textsuperscript{p} < 0.05, §§§\textsuperscript{p} < 0.01, and §§§§\textsuperscript{p} < 0.001 vs. DOCA-salt.

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in NP gene expression. In agreement with previous work, we found that DOCA-salt treatment induced the characteristic switch of MHC isoforms observed in rodents after pressure of sufficient degree and duration consisting in a relative increase in β-MHC transcript level and a reduction of the α-isof orm transcript. In contrast to the observed lack of increase of ventricular ANF gene expression after ET\textsubscript{B} blockade, it was found that the administration of A-192621 further increased β-MHC gene expression in both right and left ventricles over that found in DOCA-salt rats but α-MHC was not affected. These results suggest that ET-1 through the ET\textsubscript{B} receptor exerts a protective role on cardiac function during hemodynamic overload. Furthermore, the higher increase of β-MHC in DOCA-salt does not correlate with higher ANF transcript levels.

The effect of endothelin on interstitial cardiac fibrosis is thought to be mediated by the ET\textsubscript{B} receptor subtype provided that a selective ET\textsubscript{A} antagonist does not suppress the stimulatory effect of ET-1 on collagen synthesis (14, 21). Previous work from this laboratory showed that ET\textsubscript{A} blockade does not modify cardiac fibrosis induced by hemodynamic overload as assessed by ventricular collagen gene expression in DOCA-salt hypertensive rats (1). We found in the present work...
REFERENCES


