

## Hypertension, Diabetes Mellitus, Hypercholesterolemia, and Endothelin B Receptor-Mediated Renal Nitric Oxide Release

To the Editor:

Kakoki et al<sup>1</sup> recently reported a reduced endothelin (ET) B receptor-mediated release of nitric oxide (NO) in renal perfusates of experimental models of hypertension, diabetes mellitus, and hypercholesterolemia, as measured by H<sub>2</sub>O<sub>2</sub>-based chemiluminescence. Reduced immunostaining for endothelial ET<sub>B</sub> receptors, as measured by semiquantitative immunohistochemistry, was observed in all experimental models, whereas staining for endothelial NO synthase (eNOS) was reduced in salt-sensitive Dahl rats but not spontaneously hypertensive (SHR) or hypercholesterolemic rats. Although interesting, these observations must be interpreted with caution.

In SHR, the release of NO was not impaired, which contrasts with previously published data. eNOS activity is increased in SHR, but endothelial NO levels are reduced due to inactivation by the superoxide anion.<sup>2</sup> This discrepancy is not commented on by the authors, and the previously published work was not cited.

In experimental diabetes and hypercholesterolemia, renal eNOS expression was unchanged, while ET<sub>B</sub>-mediated renal NO release was markedly reduced. The authors propose that the reduced expression of the ET<sub>B</sub> receptor accounts for the reduction in NO release without having investigated the effect of antioxidants such as superoxide dismutase, vitamin C, or N-acetylcysteine. Finally, the authors used whole, perfused kidneys to measure NO release in the renal vein, without assessing ET<sub>B</sub> receptor and NO synthase expression in nonvascular renal tissue, which also affects NO levels in the perfusate.

Interestingly, an infusion of the ET<sub>B</sub> agonist BQ-3020 of  $<10^{-10}$  mol/L reduced renal perfusion in salt-resistant Dahl rats, whereas renal perfusion in salt-sensitive animals was increased. The response to BQ-3020 was greater in salt-sensitive animals, despite their reduced NO release and eNOS immunostaining. These conflicting observations were not explained; nor did the authors investigate other potential mechanisms, such as ET<sub>B</sub> receptor-mediated prostacyclin release.

With regard to ET-1 levels in Dahl rat plasma, these data were previously published in both salt-sensitive and salt-resistant strains in a study that included long-term treatment with an endothelin antagonist.<sup>3</sup>

ET<sub>A</sub> receptors modulate ET-1-induced vasoconstriction and cell proliferation. ET<sub>B</sub> receptors mediate ET-1 clearance in rats, and ET<sub>B</sub> blockade increases peripheral vascular resistance in humans. In patients with chronic nitric oxide deficiency, ET<sub>A</sub> selective, but not combined ET blockade, improves NO-mediated endothelial function (unpublished observation), as can be observed in mice with atherosclerosis.<sup>4</sup> Finally, chronic antagonism of the ET<sub>B</sub> receptor worsens renovascular function and structure in salt-sensitive hypertension,<sup>5</sup> an effect that is also observed in dogs with congestive heart failure.

On the basis of current knowledge regarding the ET system, the authors' statement that ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists "are more useful than ET<sub>A</sub> receptor antagonists in improving peripheral circulatory disturbances" does not hold true.

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### Response

We would like to thank Drs Barton and d'Uscio for their thoughtful comments on our article. Most pharmacological studies have suggested a normal or somehow increased release of nitric oxide (NO) in spontaneously hypertensive rats (SHR).<sup>1,2</sup> We have measured the NO released from isolated kidneys in 4 different series of SHR and Wistar Kyoto rats since 1993 and have confirmed our results.<sup>3</sup> We and others have shown that the attenuated response of uncomplicated SHR to endothelium-dependent vasodilators is due to a decrease in an endothelium-derived hyperpolarizing factor rather than to a decrease in NO itself.<sup>3</sup>

We suggested that increased endothelin (ET)-1 down-regulated the expression of the ET type B receptor (ETBR). Therefore, we measured ET-1 levels in the plasma of Dahl rats. As mentioned, our assay system may detect NO from the tubuli and macula densa as well as that from the vascular endothelium. However, the response of the NO signal to acetylcholine or BQ-3020 was very rapid and was not influenced by pretreatment with 7-nitroindazole, a neuronal NO synthase inhibitor, which suggests that most of the signal reflects endothelium-derived NO.

ETBR stimulation acts on both endothelial cells and vascular smooth muscle cells (VSMCs) and results in contrasting effects on vascular tone (ie, vasodilation and vasoconstriction in each type of cell, respectively). Changes in vascular tone induced by BQ-3020 are determined by the balance of NO release and its effects on VSMCs. The most important observation in our article, we believe, is that relatively low doses of BQ-3020 dilated normal vessels, while the same levels constricted vessels from rats with hypertension, diabetes mellitus, or hypercholesterolemia. However, the increased vasoconstrictive response in diseased animals is probably due to both reduced NO release and increased vasoconstrictive activity. Because we infused BQ-3020 intra-arterially, it is possible that BQ-3020 easily reached the endothelium, even at low doses. Higher doses of BQ-3020 may be required to stimulate ETBR in VSMCs. However, because the perfusate contained indomethacin, it is unlikely that prostacyclin was the major factor accounting for differences in vascular responses to BQ-3020.

It is still controversial as to whether ET type A receptor (ETAR) antagonists or ETAR/ETBR antagonists are beneficial for cardiovascular damage. It is clear that ETAR antagonists are beneficial in various pathological situations. Furthermore, a blockade of ETBR constricts normal vessels. The comparative study by Matsumura et al<sup>4</sup> showed the superiority of an ETAR antagonist over ETAR/ETBR antagonists in the treatment of deoxycorticosterone acetate-salt hypertension. However, the rats used showed borderline hypertension and their endothelial damage was probably minimal. In this

situation, a blockade of ETBR may result in vasoconstriction and worsen the hypertensive tissue damage. However, Cardillo et al<sup>5</sup> reported that ETAR/ETBR blockade was associated with greater vasodilation in hypertensive patients when compared with ETAR blockade; this effect was not observed in normotensive subjects. Thus, we proposed that ETAR/ETBR antagonists might be preferable for the treatment of patients with endothelial dysfunction. Further studies are required to discover if our proposal is correct for such patients.

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