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The role of ET_B receptors in normotensive and hypertensive rats as revealed by the non-peptide selective ET_B receptor antagonist Ro 46-8443

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Abstract We used Ro 46-8443, non-peptidic antagonist selective of endothelin ET_B receptors, to study the role of ET_B receptors in rat hypertension models. In normotensive rats, Ro 46-8443 decreased blood pressure, but in SHR and DOCA rats, it induced a pressor effect, due to blockade of ET_B-mediated release of nitric oxide since L-NAME prevented it. In rats rendered hypertensive by chronic L-NAME, Ro 46-8443 did not induce a pressor but depressor effect. Thus, in DOCA rats and SHR, Ro 46-8443 reveals a predominant influence of endothelial 'vasorelaxant' ET_B receptors, while in normotensive rats the prevailing role of ET_B receptors seems to be in mediating a vasoconstrictor tone.

Key words: Endothelin; ET_B receptor; Hypertensive rat; Nitric oxide; Endothelin receptor antagonist

1. Introduction

The endothelin (ET) ET_B receptor mediates complex cardiovascular effects. It is present on endothelial cells [1,2] where its activation induces endothelium-dependent relaxation via release of prostacyclin or nitric oxide or both, and may be present on smooth muscle cells to mediate direct vasoconstriction [3,4]. ET_B receptors present in certain nerve fibers might also indirectly contribute to constriction [5].

In order to evaluate the role of ET_A and ET_B receptors in pathophysiological situations, we searched for low-molecular-weight antagonists either blocking all known receptors, or selective for one of these two receptors. We have already described Ro 46-2005 and bosentan (Ro 47-0203) as the first synthetic non-peptide mixed antagonists of ET receptors [6,7]. We are now describing [8] the first non-peptide ET_B receptor selective antagonist, Ro 46-8443 ((*S*)-4-*tert*-butyl-*N*-[6-(2,3-dihydroxypropyloxy)-5-(2-methoxyphenoxy)-2-(4-methoxyphenyl)pyrimidin-4-yl]benzenesulfonamide). Because of the non-peptidic nature and low molecular weight of these compounds, their kinetics and volume of distribution is compatible with a valuable evaluation of the role of the respective receptors. Here we report on the use of Ro 46-8443 to investigate in vivo the function of ET_B receptors in normotensive and hypertensive rats. In the first part of the study, we assessed the antagonistic properties of Ro 46-8443 on the in vivo effects of the ET_B selective agonist sarafotoxin S6c. We then evaluated the hemodynamic effects of Ro 46-8443 in Wistar normotensive rats and in rats with different models of hypertension.

2. Materials and methods

2.1. Inhibition by Ro 46-8443 of ET_B-mediated responses

The inhibitory effect of Ro 46-8443 on the hemodynamic changes induced by the selective ET_B agonist sarafotoxin S6c was studied in anesthetized normal rats. Male Wistar rats (350–390 g) were anesthetized with sodium thiobutabarbital (Inactin, 125 mg/kg i.p.). The femoral artery (for blood pressure monitoring) and vein (for injection) were cannulated. After stabilization of blood pressure, Ro 46-8443 (3 or 10 mg/kg i.v.) or its vehicle (1 ml/kg of a 10% DMSO solution in water) was injected. 5 min later, increasing doses of sarafotoxin S6c were injected in a cumulative manner, each dose being given after stabilization of the effect of the previous dose on blood pressure. The hemodynamic changes induced by sarafotoxin S6c are composed of a depressor component (ET_B-mediated endothelium-dependent relaxation) and a pressor component (ET_B-mediated smooth muscle cell contraction). The combined antagonism of receptors mediating opposite actions may lead to paradoxical observations, such as absence of apparent inhibition or even potentiation of the effects of the agonist [9]. Because we had previously shown that blockade of nitric oxide synthase, which partially decreased the duration but not the amplitude of the blood pressure lowering effect of ET-1 or sarafotoxin S6c, could unmask the inhibitory effect of antagonists on the pressor component [9], N^G-nitro-L-arginine (L-NAME, 10 mg/kg i.v.) was injected 10 min before Ro 46-8443.

2.2. Effects of Ro 46-8443 in normotensive and hypertensive rats

Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), rats made hypertensive by deoxycorticosterone (40 mg), unilateral nephrectomy and salt (1% in drinking water) (DOCA rats) and rats made hypertensive by 5-day administration of L-NAME (30 mg/kg per day) in drinking water (all 320–380 g) were anesthetized with Inactin. The femoral artery and vein were cannulated for blood pressure measurements and i.v. injections, respectively. After stabilization of blood pressure, cumulative doses of Ro 46-8443 or its vehicle (10% DMSO in water) were injected in a volume of 0.5 ml/kg, each dose being given after stabilization of the effect of the previous dose on blood pressure.

2.3. Mechanism of increase in blood pressure by Ro 46-8443 in SHR

SHR were anesthetized as previously described and femoral artery and vein cannulated. In a first set of experiments, L-NAME, 10 mg/kg i.v. or its vehicle (1 ml/kg water) was injected. After stabilization of blood pressure (5–10 min), Ro 46-8443, 10 mg/kg was administered. In another set of experiments, the selective ET_A receptor antagonist BQ-123 (cyclo [D-Trp-D-Asp-Pro-D-Val-Leu]) (10 mg/kg i.v.) or its vehicle (1 ml/kg of a 1.5% sodium bicarbonate solution) was injected in place of L-NAME or water, 5 min before administration of Ro 46-8443.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. Analysis of variance for univariate repeated measures was used to assess the effect of Ro 46-8443 on dose-response curves of sarafotoxin S6c and the effect of L-NAME on pressor response to Ro 46-8443.

In all other experiments, paired or unpaired Student's *t*-test were used. A P level less than 0.05 was considered significant.

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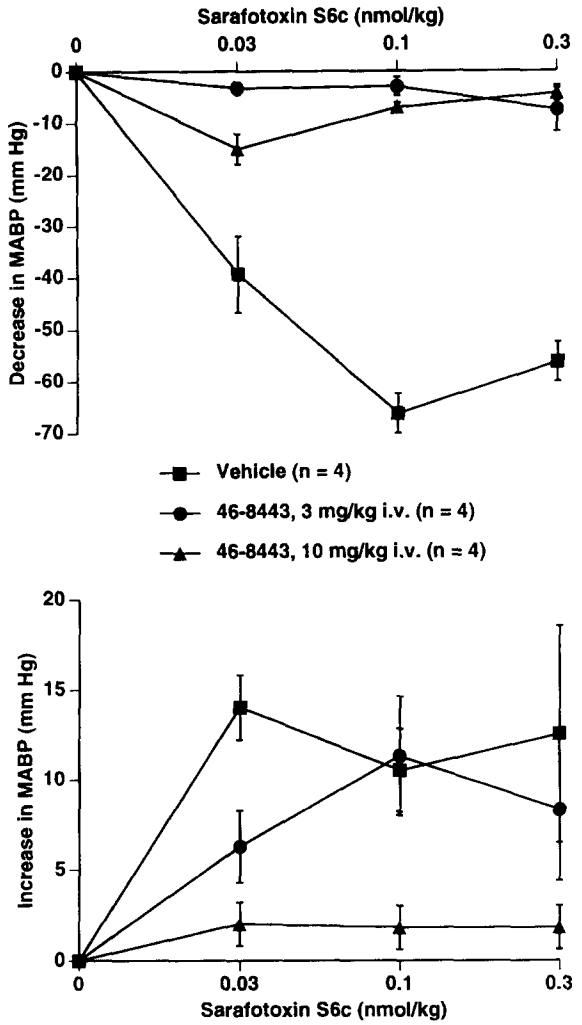

Fig. 1. Inhibition of the depressor and pressor responses to increasing doses of sarafotoxin S6c by i.v. Ro 46-8443 (3 and 10 mg/kg) in anesthetized Wistar-Kyoto rats given N^G-nitro-L-arginine (L-NAME, 10 mg/kg i.v.).

3 Results

3.1. Inhibition of ET_B-mediated responses

Sarafotoxin S6c exhibited a biphasic effect on blood pressure with a marked but transient decrease in blood pressure (depressor effect) followed by a more sustained and moderate increase (pressor effect). Ro 46-8443 (3 and 10 mg/kg) inhibited both the depressor and pressor effects of sarafotoxin S6c (Fig. 1). A dose of 10 mg/kg had a virtually complete inhibitory effect on both components.

3.2. Effects of Ro 46-8443 in normotensive and hypertensive rats

In anesthetized normotensive WKY rats (baseline mean arterial blood pressure 108 ± 3 mmHg), Ro 46-8443 had no effect on blood pressure at 3 and 10 mg/kg, while at 30 mg/kg it significantly decreased blood pressure by 22 ± 4 mmHg ($P < 0.05$) (Fig. 2). In contrast, in SHR (baseline mean artery blood pressure 169 ± 3 mmHg), Ro 46-8443 exhibited a dose-dependent increase in blood pressure, significant at 10 mg/kg ($P < 0.01$) (Fig. 2). Such a pressor effect of Ro 46-8443 was also observed in Doca rats (baseline mean arterial pressure 128 ± 20 mmHg), where doses of Ro 46-8443 of 3 mg/kg and above induced significant increases in mean arterial blood pressure (Fig. 2). In marked contrast, rats chronically treated with L-NAME (mean arterial blood pressure 147 ± 2 mmHg) responded to Ro 46-8443 by a decrease in blood pressure, significant at 30 mg/kg (Fig. 3).

3.3. Mechanism of increase in blood pressure by Ro 46-8443 in SHR

Ro 46-8443 (10 mg/kg) induced a 10 mmHg increase in blood pressure in anesthetized SHR. Administration of L-NAME (10 mg/kg) — which increased blood pressure by 36 ± 4 mmHg — completely prevented this increase in blood pressure (Fig. 4). In contrast, BQ-123 (10 mg/kg) did not modify the pressor effect of Ro 46-8443 (Fig. 5).

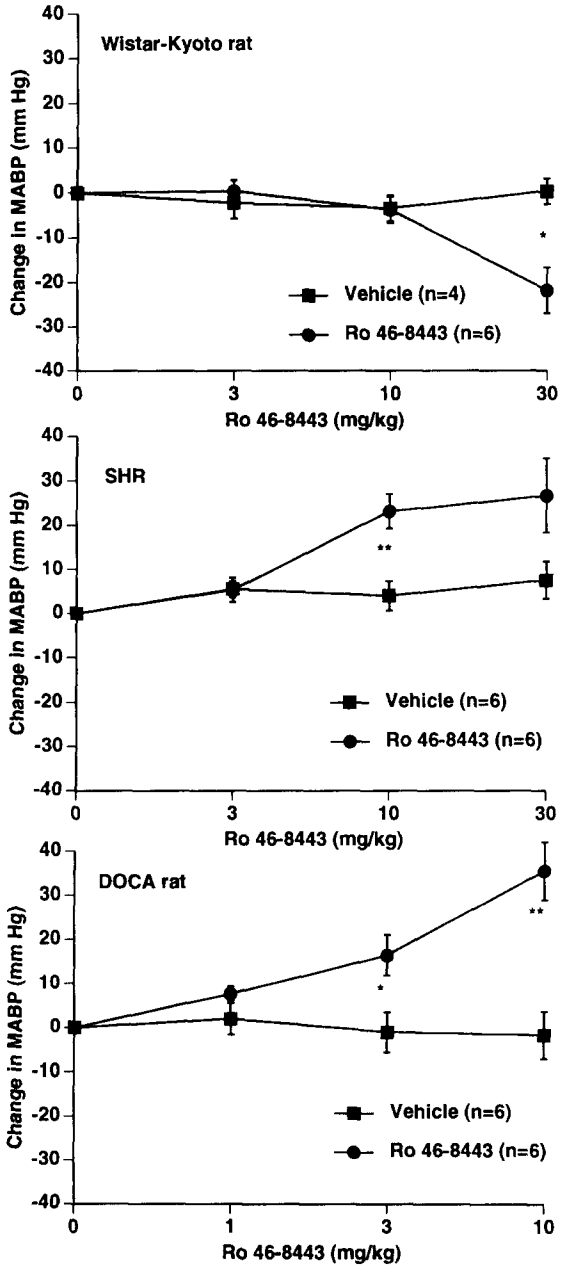

Fig. 2. Effect of i.v. Ro 46-8443 or its vehicle on mean arterial blood pressure (MABP) in anesthetized Wistar-Kyoto rats, spontaneously hypertensive rats (SHR) and DOCA hypertensive rats. $*P < 0.05$, $**P < 0.01$ compared with vehicle.

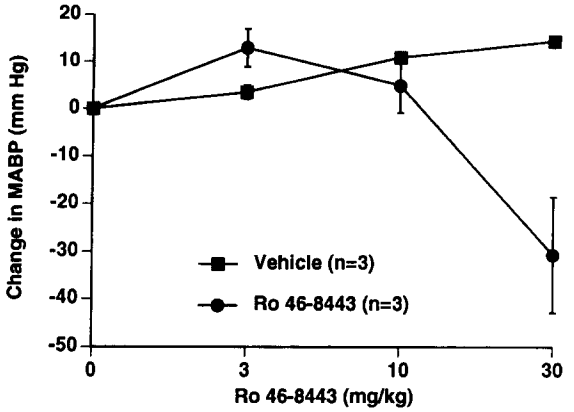

Fig. 3. Effect of i.v. Ro 46-8443 or its vehicle on blood pressure in anesthetized rats pretreated with L-NAME (30 mg/kg/day in drinking water for 5 days).

4. Discussion

The results of our study show that both in DOCA rats and in SHR, Ro 46-8443 exhibits a pressor effect which reveals a tonic influence of endothelial 'vasorelaxant' ET_B receptors, while in normotensive rats (and in rats rendered hypertensive by injection of L-NAME) the prevailing role of ET_B receptors seems to be in mediating a vasoconstrictor tone.

We have shown in a separate manuscript [8] that Ro 46-8443 is a selective antagonist of the ET_B receptor and inhibits in vitro various responses mediated by this receptor. No partial agonistic effect was detected. It is to our knowledge the first non-peptidic ET_B receptor selective antagonist described. This non-peptidic nature and its selectivity make it a particularly suitable tool for evaluating in vivo the status of ET_B receptors.

The present study shows that in vivo (as in vitro), Ro 46-8443 behaves as an inhibitor of ET_B-mediated responses. Ro 46-8443 fully inhibited the depressor — endothelium-dependent — and pressor effects of sarafotoxin S6c. We used L-NAME to prevent the nitric oxide pathway of the endothelium-dependent relaxation to sarafotoxin S6c, in order to better reveal the antagonistic effect of Ro 46-8443 on the pressor action of sarafotoxin S6c, as already described [9].

In two different rat models of hypertension, Ro 46-8443 exhibited a dose-dependent pressor effect, while it did not show such an effect in normotensive rats or in rats made hypertensive by chronic blockade of nitric oxide synthase with L-NAME. The observations made in SHR and DOCA rats suggest that the pressor effect of Ro 46-8443 might be due to the inhibition by Ro 46-8443 of the ET_B receptor-mediated release of endothelium-dependent relaxing factor(s), singularly nitric oxide. This hypothesis is confirmed by the fact that L-NAME completely abolished the pressor effect of Ro 46-8443 in SHR, showing that if Ro 46-8443 cannot inhibit the release of nitric oxide, it loses its pressor effect. Because nitric oxide plays a basal role in the modulation of blood pressure, its inhibition by Ro 46-8443 leads to an increase in blood pressure, either by direct inhibition of the release of nitric oxide or via potentiation of the effect of vasoconstrictor substances. Alternatively, another hypothesis was that the pressor effect of Ro 46-8443 in SHR could be due to an activation of ET_A receptors by an increased level of plasma ET-1. Indeed, ET receptor antagonists have been shown to increase plasma ET-1 concentrations via antagonism of ET_B receptors [10], and Ro 46-8443 itself has been shown to increase plasma ET-1 levels (data not shown). The observation that BQ-123 had no effect on the increase in blood pressure by Ro 46-8443 shows that ET_A receptors are not involved.

Overall, these findings suggest that 'dilating' ET_B receptors seem to actively modulate blood pressure in hypertension. This is in contrast with an apparent absence of basal dilating role of ET_B receptors in the normotensive state, where dilating ET_B receptors seem to play no significant role in the regulation of blood pressure, since no pressor effect of Ro 46-8443 was observed. This discrepancy may be due either to an increase of the basal level of endothelium-dependent vasodilation in situations of hypertension, as some [11] but

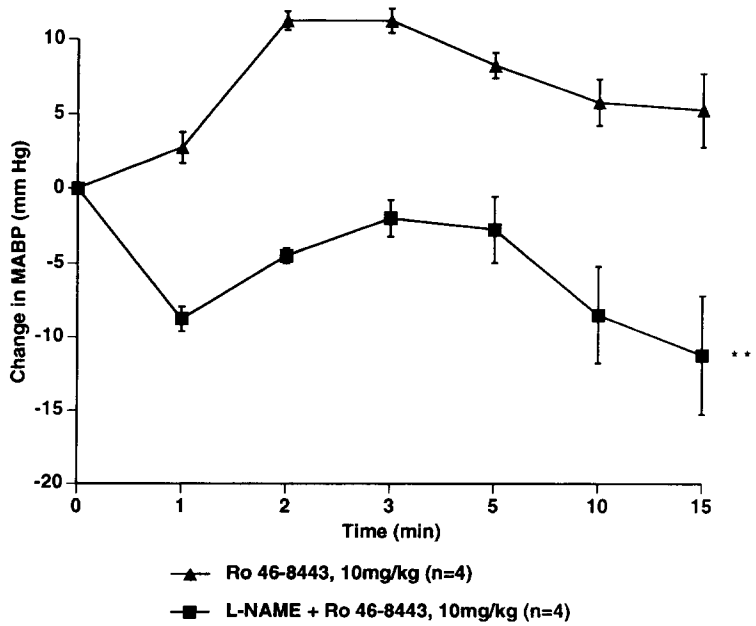

Fig. 4. Inhibition by L-NAME (10 mg/kg i.v.) of the pressor effect of Ro 46-8443 (10 mg/kg i.v.) in anesthetized SHR.

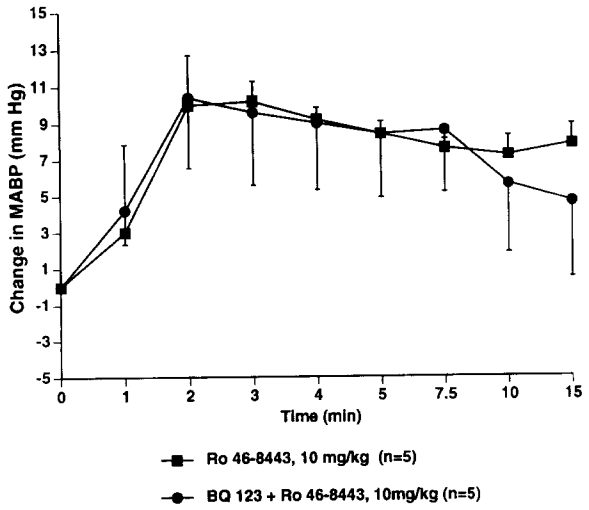

Fig. 5. Absence of effect of BQ-123 (10 mg/kg i.v.) on the pressor effect of Ro 46-8443 (10 mg/kg i.v.) in anesthetized SHR.

not others [12] have suggested, or to an up-regulation of endothelial 'vasodilating' ET_B receptors. The latter hypothesis is supported by similar results found in a rat model of pulmonary hypertension [13].

However, up-regulation of 'constricting' ET_B receptors has also been described in rat models of hypertension. Indeed, constricting ET_B receptors are up-regulated in renal arteries from SHR, as shown by an increased responsiveness to sarafotoxin S6c [14], and in SHR aorta, as shown by an increased binding of an ET_B selective ligand in aortic smooth muscle cells from SHR [15]. A contribution of ET_B receptors mediating constriction in the pathogenesis of high blood pressure in SHR is further suggested by the observation that mixed ET_A and ET_B receptor antagonism with bosentan provided a larger decrease in blood pressure in SHR as compared to selective ET_A antagonism using BQ-123. Overall, the data suggest that not only constricting ET_B receptors but also dilating ET_B receptors are up-regulated in hypertension, either via a common mechanism of regulation or by a compensatory increase in the 'moderating' endothelial ET_B receptors upon an increase in arterial pressure or shear stress. However, one can question why the present experiments using Ro 46-8443 do not reveal the contribution of the constricting receptors to blood pressure regulation in SHR but only the 'relaxing' ET_B receptors.The existence of a cross-talk between ET_A and ET_B receptors present on smooth muscle cells [16], allowing ET_A receptors to compensate upon blockade of ET_B receptors only, may provide an explanation for the lack of blood pressure lowering effect of ET_B receptor antagonists (present study, and [17]).

In normotensive rats, Ro 46-8443 exhibited no effect on blood pressure at 3 and 10 mg/kg. This is in agreement with the described absence of substantial change in blood pressure in rats treated with BQ-788 (3 mg/kg), a peptidic selective ET_B receptor antagonist [18]. In contrast, Ro 46-8443 exhibited a significant depressor effect at the higher dose of 30 mg/kg. This suggests a prevalent contribution of constricting ET_B receptors in normotensive rats. The need for a higher dose of Ro 46-8443 as compared to the doses leading to pressor effects in SHR and DOCA rats may be due to the need for Ro 46-8443 to reach the smooth muscle cells for blocking constricting ET_B receptors and eliciting this depressor effect in normotensive rats.

In conclusion, our data show that ET_B receptor blockade with Ro 46-8443, which may lower blood pressure in normotensive rats, in contrast leads to an increase in blood pressure in spontaneously hypertensive and DOCA hypertensive rats, revealing an upregulation of relaxing ET_B receptors in situations of hypertension. We have previously shown that Ro 46-8443 was able to prevent neurogenic inflammation in the dura mater of the rat [19]. If this model can be used as a model of migraine, as suggested by Moskowitz [20], then Ro 46-8443 and other ET_B receptor antagonists could be potentially useful as anti-migraine agents. However, our findings from the present study suggest that ET_B receptor selective antagonists raise blood pressure in rat models of hypertension. If this finding applies to man, ET_B receptor selective antagonists should be used with caution in patients with cardiovascular diseases.

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