Effects of benazepril, an angiotensin-converting enzyme inhibitor, combined with CGS 35066, a selective endothelin-converting enzyme inhibitor, on arterial blood pressure in normotensive and spontaneously hypertensive rats

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ABSTRACT

Continuous intra-arterial administration of a selective endothelin-converting enzyme (ECE) inhibitor CGS 35066 at a dose of 30 mg/kg decreased the mean arterial blood pressure (MABP) in conscious unrestrained normotensive rats and spontaneously hypertensive rats (SHRs). At that dose, the magnitude of the antihypertensive effects was greater in SHRs than in normotensive rats. Additional administration of an angiotensin-converting enzyme (ACE) inhibitor benazepril (lotensin) further reduced MABP in normotensive rats and completely blocked hypertension in SHRs. However, when the selective ECE inhibitor was subsequently removed, blood pressure was less inhibited in normotensive rats whereas it remained strongly inhibited in SHRs by the ACE inhibitor alone. These results imply that simultaneous treatment with benazepril and CGS 35066 gave additive antihypertensive effects in normotensive rats but not in SHRs, when both compounds were administered at a dose of 30 mg/kg. Our results suggest that: (i) the endothelin (ET) system together with the renin–angiotensin system contribute to the maintenance of blood pressure in normal healthy rats; (ii) while an ECE inhibitor acts as an antihypertensive agent on its own, the sole efficacy of ACE inhibitor at that dose is sufficient to block MABP without the participation of the ET system in SHR.

INTRODUCTION

CGS 35066 \((\alpha R)-x[(phosphonomethyl)amino]-dibenzo-furan-3-propanoic acid\) is a potent non-peptidic and selective endothelin-converting enzyme (ECE) inhibitor, which was synthesized by replacing the tetrazol in CGS 34043, a dual inhibitor of ECE and neutral endopeptidase (NEP), with a carboxylic acid [1–3]. It has an IC_{50} of 22 nM against recombinant human ECE-1 and an IC_{50} of 2.3 μM against rat kidney NEP. In normotensive, con-

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Abbreviations: ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; ET, endothelin; MABP, mean arterial blood pressure; NEP, neutral endopeptidase; SHR, spontaneously hypertensive rat.

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conscious catheterized healthy rats, CGS 35066, administered intravenously at 0.3, 1, 3 and 10 mg/kg, was reported to block the pressor responses induced by intravenous administration of big endothelin-1 (ET-1) (0.3 nmol/kg) by 61, 78, 93 and 98% (at 30 min), and by 29, 63, 63 and 84% (at 120 min) respectively. The effect was selective since the pressor effect induced by exogenous angiotensin I was unaffected [4]. In addition, this novel compound was found to attenuate neointimal proliferation of the rat carotid artery following balloon angioplasty (D. Pham, F. Thaveau, B. Ayach, A. Y. Jeng, E. Escher, S. Plante and B. Battistini, unpublished work).

In the present experiments, we assessed whether this potent and selective ECE inhibitor was capable of reducing the mean arterial blood pressure (MABP) in a genetic hypertensive model, the spontaneously hypertensive rat (SHR). While there is no report in the literature about combined use of ECE and angiotensin-converting enzyme (ACE) inhibitors, there is one publication that showed potentiation of the antihypertensive effects of a non-selective endothelin ET\(_a\)/ET\(_b\)-receptor antagonist by an angiotensin 1 receptor antagonist (losartan) in three rat models of hypertension [5]. Thus, we examined the effect of CGS 35066, administered simultaneously with the ACE inhibitor benazepril [6,7], on blood pressure control in both the normotensive rats and SHRs.

### MATERIALS AND METHODS

#### Animals and surgical implantation

Healthy male normotensive Sprague–Dawley rats and young SHRs (8–12 weeks old; 400 ± 25 g) were obtained from Charles River (St Constant, QC, Canada). They were fed a normal diet and kept according to the Canadian Council of Animal Care and local guidelines. A vascular catheter was implanted in the right femoral artery to constitute instrumented, unrestrained and conscious models, as described previously [8].

#### Experimental protocol and direct blood pressure measurement

Rats were divided into four groups (\(n = 5–10\) animals/group): Group 1, non-treated normotensive Sprague–Dawley rats; Group 2, non-treated SHRs (both controls were infused intra-arterially with sterile saline); Group 3, treated Sprague–Dawley rats; Group 4, treated SHRs. All groups were kept for 19 days.

The following protocol was initiated 4 days after surgical implantation of a vascular catheter and adaptation wearing the elastomer jacket. Unrestrained conscious rats were infused for 3 days with vehicle (0.25 M \(\text{NaH}_2\text{CO}_3\) in sterile saline; 6 ml/day, intra-arterially). The rats were then infused for an additional 3 days with CGS 35066 (30 mg·kg\(^{-1}\)·day\(^{-1}\) intra-atrially), then with CGS 35066 plus benazepril (30 mg·kg\(^{-1}\)·day\(^{-1}\) intra-atrially) for 3 days, than CGS 35066 was removed to leave only benazepril at the same dose for a further 3 days, to finally remove the remaining drug and infusing only saline for 3 days (a total period of 15 consecutive days).

The MABP of each animal measured at 2.00 PM was used for statistical comparison. The arterial vascular catheter was connected to a pressure transducer (Harvard Apparatus, St Laurent, QC, Canada), and signals were recorded continuously on a computerized system (PO-NE-MAH Systems, Gould Instruments, Simsbury, CT, U.S.A.).

#### Statistical analysis

Results are given as means ± S.E.M. For comparisons within groups, a randomized block design was applied using two factors defined for the analysis: the subject effect and the time period effect. Comparison between groups was performed by a three-way ANOVA with a blocking factor representing subjects. Interaction between the time period factor was used to compare groups. When interaction was significant for parameters, comparisons at different time periods were analysed using Student’s paired \(t\) tests. The normality and variance assumptions were met for almost all data. All analyses were conducted using the statistical package SAS (SAS Institute Inc, Cary, NC, U.S.A.).

#### RESULTS

MABP was stable in both control groups throughout the experiments; the values ranged from 107–114 mmHg in Sprague–Dawley rats and from 160–170 mmHg in SHRs (Figure 1a). Treatment with the ECE inhibitor resulted in a decrease in MABP in both normotensive and hypertensive rats. In absolute values (mmHg), the effect in SHRs was 2-fold higher than that in normotensive animals (Figure 1b), but both groups had the same percentage reduction from baseline levels. Addition of the ACE inhibitor, with the ECE inhibitor, further reduced MABP by 32% and 42% in normotensive rats and SHRs respectively. The mean reduction in MABP in SHRs was twice that of the normotensive rats. When the infusion of the ECE inhibitor was terminated, MABP increased toward the baseline levels in Sprague–Dawley rats, whereas it was not affected in SHRs after 3 days of treatment. Upon the removal of both inhibitors, the MABP of the Sprague–Dawley rats returned to baseline levels, while it was still significantly attenuated in the SHRs, when compared with the baseline values (Figures 1a and 1b).
Figure 1  Effects of ACE and ECE inhibitors on the MABP of normotensive rats and SHRs

Rats were first infused with saline for 3 days. The rats were then infused for an additional 3 days with CGS 35066 (30 mg kg⁻¹ day⁻¹ intra-arterially), then with CGS 35066 plus benazepril (30 mg kg⁻¹ day⁻¹ intra-arterially) for 3 days, then CGS 35066 was removed to leave only benazepril at the same dose for a further 3 days, to finally remove the remaining drug and infusing only saline for 3 days (a total period of 15 consecutive days). (a) Points and error bars represent the mean and S.E.M. of MABP respectively. * P < 0.05, significantly different over time. (b) Averaged reduction of the MABP from baseline. † P < 0.05, significantly different over time from the vehicle-treated group; ‡ P < 0.05, significantly different from the previous treatment groups. ECEi, endothelin-converting enzyme inhibitor; ACEi, angiotensin-converting enzyme inhibitor; SD, Sprague–Dawley.

DISCUSSION

The use of distinct molecules to inhibit dual, or even triple pathways for the treatment of hypertension is not a novel idea. The emergence of a potent NEP inhibitory activity in combination with ACE inhibitory activity into one distinct molecule constitutes a good example of vasopeptidase inhibition [9]. The development of novel class of molecules introduces a new paradigm in circulatory modulation. There are major advantages in dual and triple inhibition, e.g. the ability to lower blood pressure more effectively and to lower the dose and, consequently, the potential side effects, irrespective of renin or volume status.

CGS 35066, a selective ECE inhibitor, represents a novel class of molecules that targets the humoral ET system [1–3]. We found that it decreased MABP in both normotensive rats and SHRs. Conversely, the ACE inhibitor was much more potent in genetically hypertensive rats than in normotensive rats. A combination of the two agents provided additive effects in normotensive rats, suggesting that both the ET and angiotensin systems are involved in normal blood pressure regulation. However, the ACE inhibitor alone was potent enough to strongly inhibit the elevated blood pressure in SHRs. The high dose that we have used may have masked the effect of an ET-related component in SHRs.

In the literature, ET receptor antagonists have been reported to be effective modulators for the lowering of arterial blood pressure in rats [5,10], but they revealed different degrees of effectiveness in various hypertensive models. Our observations are different from those reported in Wistar Kyoto and Dahl salt-resistant rats where there were no effects using a non-selective ET_A/ET_B receptor antagonist [5]. Discrepancies are also noted in comparison with the three rat models of hypertension in that same study [5]. The discrepancies between these results may be explained by: (i) the higher dose used in our experiments (30 mg kg⁻¹ day⁻¹) in comparison to those used in the SHR, stroke-prone SHR and Dahl salt-sensitive hypertensive rats (3 and 10 mg/kg), where an angiotensive 1 receptor antagonist did augment the antihypertensive activity of a mixed ET_A/ET_B receptor antagonist [5]; (ii) the way the drugs were administered (slow continuous intra-arterial infusion versus per os); and (iii) the age of the rats [our SHRs were young (<12 weeks) compared with the 23–26-week-old SHRs in other studies].

Since ET_B receptor blockade participates in the depressor response of non-selective ET_A/ET_B receptor antagonist [5], the use of an ECE inhibitor, which blocks the biosynthesis of ET could be as effective. The role of endothelial ET_A receptor (ET_A-mediated release of vasodilators) and vascular smooth muscle ET_B receptor (ET_B-mediated vasoconstriction) in blood pressure regulation has yet to be fully resolved.

In conclusion, the present results support the role of the ET system in vascular and blood pressure regulation of both normotensive rats and SHRs. Other experiments using lower doses of various combinations of ECE,
ACE, and even NEP inhibitors, are needed to assess blood pressure control in the SHR and other models. These experiments are also needed before a simultaneous administration of ACE and ECE inhibitors could be considered to have major benefits for the treatment of hypertension in situations where other therapy for the regression of cardiac hypertrophy or decrease in morbidity and mortality is not adequate, or fully effective.

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