Endothelin B receptors located on the endothelium provide cardiovascular protection in the hamster

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ABSTRACT

Endothelins (ETs) act through two receptors, namely ET_A and ET_B. In the cardiovascular system, the activation of both receptors leads to vasoconstriction. However, ET_B receptors also mediate endothelium-dependent vasodilatation and clearance of plasma ET-1. With regard to these latter properties, we wanted to assess the contribution of ET_B receptors and the effects of selective and mixed ET receptor blockade on vascular tone in control Syrian Golden hamsters and in Bio 14.6 cardiomyopathic hamsters after bolus injection of ET-1 and IRL-1620, a selective ET_B agonist. In 12-week-old anaesthetized control hamsters, ET-1 (0.5 nmol/kg) induced a sustained pressor response which was only partly reduced by the selective ETA receptor antagonist BQ-123, suggesting a contribution of ET_B receptor activation to the vasoconstrictive effects of ET-1. This was confirmed by injection of the selective ET_B receptor agonist IRL-1620. However, the pressor response to this agonist was always preceded by a transient vasodilatation, indicating activation of endothelium-located ET_B receptors. When the selective ET_B receptor antagonist BQ-788 was administered, the hypotensive phase following IRL-1620 injection was abolished. Interestingly, BQ-788 or a mixture of BQ-788 and BQ-123 significantly potentiated the pressor responses to ET-1. In 12-week-old Bio 14.6 cardiomyopathic hamsters, ET-1 and IRL-1620 induced haemodynamic responses similar to those observed in control hamsters, although the IRL-1620-induced pressor increase was lower. No difference in cardiac prepro ET-1 mRNA expression was observed between the two strains of hamsters. In conclusion, we suggest that endothelium-located ET_B receptors are involved in the physiological antagonism of ET-dependent protracted pressor effects, and thus may play a protective role in both normal hamsters and those with cardiomyopathy.

INTRODUCTION

The endothelins (ET-1, ET-2 and ET-3) are potent vasoconstrictor peptides that have been implicated in numerous physiological and pathophysiological cardiovascular processes [1]. In humans, ET-1 induces its physiological actions through two G-protein-coupled receptors, namely ET_A and ET_B. While ET_A receptors have a greater affinity for ET-1 and ET-2, leading predominantly to vasoconstriction, ET_B receptors express a similar affinity for all three isopeptides, triggering endothelium-dependent vasodilatation and clearing ETs from blood circulation [2,3]. Interestingly, Cowburn et al. [7] demonstrated that ET_B-mediated vasoconstriction is increased in patients with heart failure. Nevertheless, Strachan et al. [8] reported that the systemic blockade of ET_B receptors increases peripheral vascular resistance in healthy men, suggesting that the balance of effects of endogenous ET-1 at the vascular ET_B receptor favours vasodilatation.

Key words: blood pressure, ET_A and ET_B receptors, reverse transcriptase–PCR.
Abbreviations: ET, endothelin; RT, reverse transcriptase.
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The Bio 14.6 cardiomyopathic hamster is an interesting experimental model because it develops the major characteristics observed in humans during progressive cardiomyopathy over a predictable time course [9]. Yamauchi-Kohno et al. [10] reported a marked increase in prepro-ET mRNA in the cardiac muscle of cardiomyopathic hamsters, and demonstrated the beneficial effects of a highly selective ET<sub>B</sub> receptor antagonist in reversing the observed cardiodilatation and improving the life span of Bio 14.6 cardiomyopathic hamsters.

In an attempt to assess the contribution of ET<sub>B</sub> receptors to vascular tone in control Syrian Golden hamsters and in Bio 14.6 cardiomyopathic hamsters, we have studied the acute pressor responses to exogenously administered non-selective and selective ET<sub>B</sub> receptor ligands (ET-1 and IRL-1620 respectively) in these animals. Moreover, we have investigated the effects of selective ET<sub>A</sub> and ET<sub>B</sub> receptor antagonists (administered separately or combined) to determine whether ET<sub>B</sub> receptors should be occupied by an antagonist to interfere with the activity of the endogeneous agonist ET-1.

**METHODS**

Groups of 12-week-old Syrian Golden hamsters (Charles River, Montréal, QC, Canada) and Syrian Bio 14.6 cardiomyopathic hamsters (Biobreeders, Fitchburg, MA, U.S.A.) were anaesthetized with ketamine (87 mg/5 kg, intramuscular). The left external jugular vein and right external carotid artery were cannulated (PE-10 catheters) for, respectively, drug administration and recording of both mean arterial blood pressure and heart rate using a blood pressure analyser (model 200; Micro-Med, Louisville, KY, U.S.A.). Responses to ET-1 or IRL-1620, a selective ET<sub>B</sub> receptor agonist, were followed over 45 min. When specified, agonist administration was preceded by an intravenous injection of antagonists, given 5 min previously (see below). The care of animals and all the research protocols conformed to the guiding principles for animal experimentation, as enunciated by the Canadian Council on Animal Care, and were approved by the Ethical Committee on Animal Research of the Université de Sherbrooke.

ET-1 and IRL-1620 were dissolved in PBS, whereas BQ-123, a selective ET<sub>A</sub> receptor antagonist [11], and BQ-788, a selective ET<sub>B</sub> receptor antagonist [12], were dissolved in PBS/5% (v/v) DMSO. All drugs were purchased from American Peptide Company, Inc. (Sunnyvale, CA, U.S.A.), except BQ-123, which was synthesized in our laboratory by Dr Witold Neugebauer.

Cardiac mRNA expression was assessed after extraction and reverse transcriptase–PCR (RT-PCR) using avian myeloblastosis virus reverse transcriptase (Roche Diagnostics, Laval, QC, Canada) and Taq DNA polymerase (Fisherbrand, Nepean, ON, Canada) followed by migration on ethidium bromide-stained agarose gels according to Honoré et al. [13]. Specific primers for glyceraldehyde-3-phosphate dehydrogenase and preproET-1 were used for the PCR [10].

Results are presented as means ± S.E.M. for n experiments. The unpaired Student’s t test or Dunnnett’s test for multiple comparisons were applied for assessment of statistical significance, since studied responses to various agonists were poorly reversible. P < 0.05 was considered significant.

**RESULTS**

**Implication of ET<sub>B</sub> receptors in the pressor effects of ET-1**

Anaesthetized control Syrian Golden hamsters exhibited an average mean arterial blood pressure of 111.5 ± 1.4 mmHg (n = 53).

Figure 1(a) illustrates typical time-related pressor responses to the intravenous injection of ET-1 (0.5 nmol/kg) and IRL-1620 (1 nmol/kg). ET-1 induced a sustained increase in blood pressure that lasted for more than 30 min. The selective ET<sub>B</sub> receptor agonist IRL-1620 also triggered a pressor response (Figure 1a). However, this effect was preceded by a transient vasodilatation within the first 1 min after injection of the peptide. For clarity, this transient response will be described in this study as phase 1, whereas the sustained response will be described as phase 2.

![Figure 1](image-url)
Figure 2 Pharmacological pressor responses to ET-1 and IRL-1620 in Bio 14.6 hamsters
(a) ET-1 (0.5 nmol/kg) induced similar increases in mean arterial blood pressure (MAP) in 12-week-old Syrian Golden hamsters (SGH) and Bio 14.6 cardiomyopathic hamsters (CMH). (b) Conversely, IRL-1620 (1 nmol/kg) triggered a significantly reduced phase 2 pressor response in Bio 14.6 cardiomyopathic hamsters as compared with controls. Values represent means ± S.E.M. of 4–11 experiments. Significance of difference: *P < 0.05.

Figure 3 Cardiac expression of preproET-1 mRNA in control and Bio 14.6 hamsters
Shown is a typical representative ethidium bromide-stained gel of preproET-1 (ppET-1) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA expression in cardiac tissue from 12-week-old Syrian Golden hamsters (SGH) and Bio 14.6 cardiomyopathic hamsters (CMH) after RT-PCR. RT was included (+) or omitted (−), and a 100 bp DNA ladder is indicated (lane L).

In another set of experiments, the selective ET₁ receptor antagonist BQ-788 (0.25 mg/kg) significantly potentiated this pressor increase. Interestingly, a mixture of both antagonists had a similar effect to BQ-788 alone (Figure 1b). Otherwise, BQ-123 did not alter the response to the selective ET₁ receptor agonist IRL-1620 (control: phase 1, −9.2 ± 2.6 mmHg; phase 2, 26.3 ± 1.4 mmHg; BQ-123: phase 1, −8.6 ± 7.9 mmHg; phase 2, 22.8 ± 2.2 mmHg; n = 4–11), whereas BQ-788 blocked the depressor transient response and reduced the sustained increase in blood pressure triggered by this agonist (phase 1, 8.5 ± 1.5 mmHg; phase 2, 20.9 ± 2.1 mmHg; n = 10–11; P < 0.05).

Pressor effects of ET-1 and IRL-1620 in the Bio 14.6 cardiomyopathic hamster model
The Bio 14.6 cardiomyopathic hamsters had an average basal mean arterial blood pressure of 91.8 ± 3.2 mmHg (n = 9), which is significantly lower than that reported for control Syrian Golden hamsters, as indicated above (P < 0.05). In this pathophysiological experimental model, the pressor effect of ET-1 did not differ from that in controls (Figure 2a). In contrast, although the first depressor response phase to IRL-1620 was not altered in the Bio 14.6 cardiomyopathic hamster model, the second sustained pressor phase was significantly reduced in this model when compared with controls (Figure 2b).

Cardiac expression of preproET-1 mRNA in control and Bio 14.6 hamsters
As illustrated in Figure 3, amplification of cDNA coding for preproET-1 by RT-PCR resulted in fragments of the expected size (327 bp). Interestingly, preproET-1 expression did not vary between control and Bio 14.6 cardiomyopathic hamsters. The integrity of the mRNA and the efficiency of reverse transcription were confirmed by amplification of the same samples using specific primers for glyceraldehyde-3-phosphate dehydrogenase (Figure 3).

DISCUSSION
In the present study, we have shown that ET₁ receptors can mediate transient vasodilatation as well as a sustained vasoconstrictor pressor response in the Syrian Golden hamster. These data suggest that the Syrian Golden hamster is an adequate experimental model in which to study the dual effects of ET₁ receptors on the cardiovascular system. Indeed, in the Syrian Golden hamster and as reported in human subjects, activation of ET₁ receptors by ET-1 mediates both vasodilatation and vasoconstriction [6]. Furthermore, our present study demonstrates that endothelium-located ET₁ receptors are important in regulating the effects of the ET pathway, as blockade of these receptors results in an increase in blood pressure after ET-1 injection. Although displace-
ment of ET-1 from clearance receptors cannot be totally excluded as an explanation for this observation, the lack of effect of BQ-123 on the IRL-1620-induced pressor increase argues for a direct effect of the selective ET\textsubscript{B} receptor agonist on vascular smooth muscle cells. The increase in blood pressure observed after dual antagonism may largely be explained by blockade of endothelial ET\textsubscript{A}-receptor-dependent NO release. Indeed, Verhaar et al.\cite{Verhaar1999} have reported that vasodilatation due to BQ-123 infusion results in large part from increased NO generation. Our results thus highlight the sensitivity of endothelium-located ET\textsubscript{B} receptor blockade in the regulation of ET-1-induced pressor effects.

The Bio 14.6 cardiomyopathic hamsters were studied at 12 weeks old because no clinical signs of cardiomyopathy are detectable at this early age, although histological lesions are developing\cite{Gertz1972}. We report here that ET-1 pressor responses are not altered in young Bio 14.6 cardiomyopathic hamsters as compared with their age-matched controls. Moreover, these cardiomyopathic hamsters do not show an increased cardiac preproET-1 mRNA level. These results indicate that the cardiac preproET-1 level and the contractile cardiovascular apparatus that mediates the actions of ET-1 are unaffected at 12 weeks of age in the Bio 14.6 cardiomyopathic hamster model. However, our results also illustrate that the increase in blood pressure induced by IRL-1620 was significantly reduced in these cardiomyopathic hamsters. Whether ET\textsubscript{B} receptors are down-regulated in the vascular smooth muscle cells of these animals remains to be determined. Nevertheless, these data confirm a lesser contribution of these receptors in the ET-1-induced pressor effects. Interestingly, after IRL-1620 injection, the transient vasodilatation phase was still present in the Bio 14.6 cardiomyopathic hamsters, suggesting that ET\textsubscript{A} receptors mediating NO release, located on the endothelium, are functionally active. This last point is of particular relevance, as mentioned above, with regard to the physiological role of the receptor in the Syrian Golden hamster. The function of this receptor should thus be preserved from antagonist blockade, particularly in end-stage pathologies associated with cardiac weakness.

In conclusion, we have shown that endothelium-located ET\textsubscript{B} receptors have an important role in the physiological antagonism of ET-dependent protracted pressor effects. We suggest that these receptors may play a protective role in normal hamsters as well as in Bio 14.6 congeners.

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