The Journal of Experimental Biology 208, 3739-3746
Published by The Company of Biologists 2005
doi:10.1242/jeb.01834

Endothelin-1 causes systemic vasodilatation in anaesthetised turtles (*Trachemys scripta*) through activation of ET_B-receptors

Nini Skovgaard[1,*], Daniel E. Warren[1,2], Donald C. Jackson[2] and Tobias Wang[1]

[1]*Department of Zoophysiology, Institute of Biological Sciences, University of Aarhus, Denmark* and [2]*Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI 02912, USA*

*Author for correspondence (e-mail: nini.jensen@biology.au.dk)

Accepted 15 August 2005

Summary

The effects of endothelin-1 (ET-1) on systemic and pulmonary circulation were investigated in anaesthetised freshwater turtles (*Trachemys scripta*) instrumented with arterial catheters and blood flow probes. Bolus intra-arterial injections of ET-1 (0.4–400 pmol kg^{-1}) caused a dose-dependent systemic vasodilatation that was associated with a decrease in systemic pressure (P_{sys}) and a rise in systemic blood flow ($\dot{Q}_{sys}$), causing systemic conductance (G_{sys}) to increase. ET-1 had no significant effects on the pulmonary vasculature, heart rate (f_H) or total stroke volume ($V_{S_{tot}}$). This response differs markedly from mammals, where ET-1 causes an initial vasodilatation that is followed by a pronounced pressor response. In mammals, the initial dilatation is caused by stimulation of ET_B-receptors, while the subsequent constriction is mediated by ET_A-receptors. In the turtles, infusion of the ET_B-receptor agonist BQ-3020 (150 pmol kg^{-1}) elicited haemodynamic changes that were similar to those of ET-1, and the effects of ET-1 were not affected by the ET_A-antagonist BQ-610 (0.15 μmol kg^{-1}). Conversely, all effects of ET-1 were virtually abolished after specific ET_B-receptor blockade with the ET_B-antagonist BQ-788 (0.15 μmol kg^{-1}). The subsequent treatment with the general ET-receptor antagonist tezosentan (15.4 μmol kg^{-1}) did not produce effects that differed from the treatment with ET_B-antagonist, and the blockade of ET-1 responses persisted. This present study indicates, therefore, that ET_B-receptors are responsible for the majority of the cardiovascular responses to ET-1 in *Trachemys*.

Key words: turtle, *Trachemys*, reptile, blood flow, blood pressure, systemic circulation, pulmonary circulation, endothelin, ET_A-receptor, ET_B-receptor.

Introduction

Vascular tone is regulated by neuronal and humoral signals that, in concert with local regulatory mechanisms, provide the means for controlling blood flow distribution to various circulatory beds. Many of the local regulatory mechanisms are associated with the endothelium, which releases vasodilators such as nitric oxide (NO) and prostacyclin as well as vasoconstrictors such as endothelin-1 (ET-1) (Mateo and Artinaño, 1997; Alonso and Radomski, 2003). These mechanisms are ancient, as evidenced from their presence in bony and cartilaginous fishes (Olson et al., 1991; Evans et al., 1996; Hoagland et al., 2000), but very little is known about their role in non-mammalian tetrapods. In mammals, there are three endothelins (ET-1, ET-2 and ET-3), each of which consists of 21 amino acids, which are constitutively expressed in various tissues. It has been proposed that a gene duplication of the ancestral gene encoding for ET gave rise to ET-1 and ET-3 and that subsequent gene duplication, possibly within the mammalian lineage, of the ancestral ET-1 resulted in ET-2 (Landan et al., 1991; Platzack et al., 2002). The structure of ET-1 is highly conserved among tetrapods, with an identical amino acid sequence in frogs, alligators and humans (Wang et al., 2000; Platzack et al., 2002).

ET-1 is regarded as one of the most potent vasoconstrictors in mammals (Yanagisawa et al., 1988, 1989), and ET-1 also exerts cardiovascular effects in ectothermic vertebrates (Olson et al., 1991; Poder et al., 1991; Wang et al., 1999, 2000; Hoagland et al., 2000; Platzack et al., 2002). In fish and mammals, infusion of ET-1 normally gives rise to an initial vasodilatation that is followed by a long-lasting vasoconstriction (Yanagisawa, 1989; Olson et al., 1991). However, in the alligator, the initial vasodilatation is very pronounced and long-lasting, whereas the subsequent constriction is small and only prevalent after large dosages (Platzack et al., 2002). Thus, it seems that the cardiovascular actions of ET-1 differ markedly between reptiles and mammals (Platzack et al., 2002). Reptiles, however, are a phylogenetically very diverse group with large differences in cardiovascular structure and function (Page, 2000). More studies on other groups of reptiles are, therefore, required to reveal whether the differences between alligators and

mammals apply to all reptiles. ET-1 has been shown to cause a marked contraction of isolated vascular rings of pulmonary and systemic arches from turtles (Poder et al., 1991). These vessels, however, contribute little to vascular resistances.

ET-1 is primarily expressed and released from the vascular endothelium and acts in a paracrine fashion on ET_A- and ET_B-receptors that are located on the vascular smooth muscle and the endothelium (Yanagisawa, 1989; Miyauchi and Masaka, 1999; Masaki, 2004). In mammals, stimulation of ET_A-receptors, normally located within the smooth muscle, causes constriction. Stimulation of ET_B-receptors within the endothelium leads to vasodilatation, through the release of nitric oxide, whereas stimulation of ET_B-receptors within the smooth muscle causes constriction (Mateo and Artiñano, 1997; Masaki, 2004). In mammals, the initial dilator response to ET-1 is normally ascribed to stimulation of ET_B-receptors whereas the pressor response is ascribed to stimulation of ET_A-receptors. The role of these two different receptors has not been studied in reptiles, but ET_B-receptors have been located in various tissues of lizards (De Falco et al., 2002).

In the present study, we describe the effects of ET-1 on the systemic and pulmonary vasculature in anaesthetised turtles (*Trachemys scripta*). The cardiovascular system of this turtle is one of the most well-studied amongst reptiles (Hicks et al., 1998), but very little is known about the role of endothelium-derived factors (see Crossley et al., 2000). Through infusion of a selective ET_B-agonist, ET_B- and ET_A-specific antagonists and the general ET-antagonist tezosentan, we also assess which of the two receptor types is responsible for the haemodynamic changes caused by ET-1.

Materials and methods

Experimental animals

Freshwater turtles (*Trachemys scripta* Gray) of both sexes (1.12±0.10 kg, mean ± S.E.M., N=12) were obtained from Lemberger Inc. (Oshkosh, WI, USA) and air-freighted to Aarhus University. The animals were housed in fibreglass tanks containing freshwater heated to 28°C and had access to dry platforms under heating lamps, allowing for behavioural thermoregulation. All animals appeared healthy and were fed fish several times a week, but food was withheld for several days prior to experiments. All experiments were carried out by authorised investigators according to Danish Federal Regulations.

Surgery and instrumentation

The turtles were anaesthetised with an intramuscular injection of sodium pentobarbital (Mebumal; 50 mg kg^{-1}), which was supplemented by an additional dose (25 mg kg^{-1}) if reflexes persisted after 1 h. The animals were tracheostemised for artificial ventilation at 15 breaths min^{-1} and a tidal volume of 50 ml kg^{-1} using a Harvard Apparatus mechanical ventilator (Cambridge, MA, USA). To maintain normal acid–base status, the animals were ventilated with a gas mixture of 3% CO_2 (balance room air) prepared by a Wösthoff gas mixing pump (Bochum, Germany).

A bone saw was used to expose the central blood vessels by removing a 5×5 cm piece of the plastron. The left carotid artery was occlusively cannulated with a PE50 catheter filled with heparinised saline, while the left pulmonary artery was non-occlusively cannulated with an intravenous catheter (Terumo Surflo, Leuven, Belgium) using the Seldinger technique (White et al., 1989). All

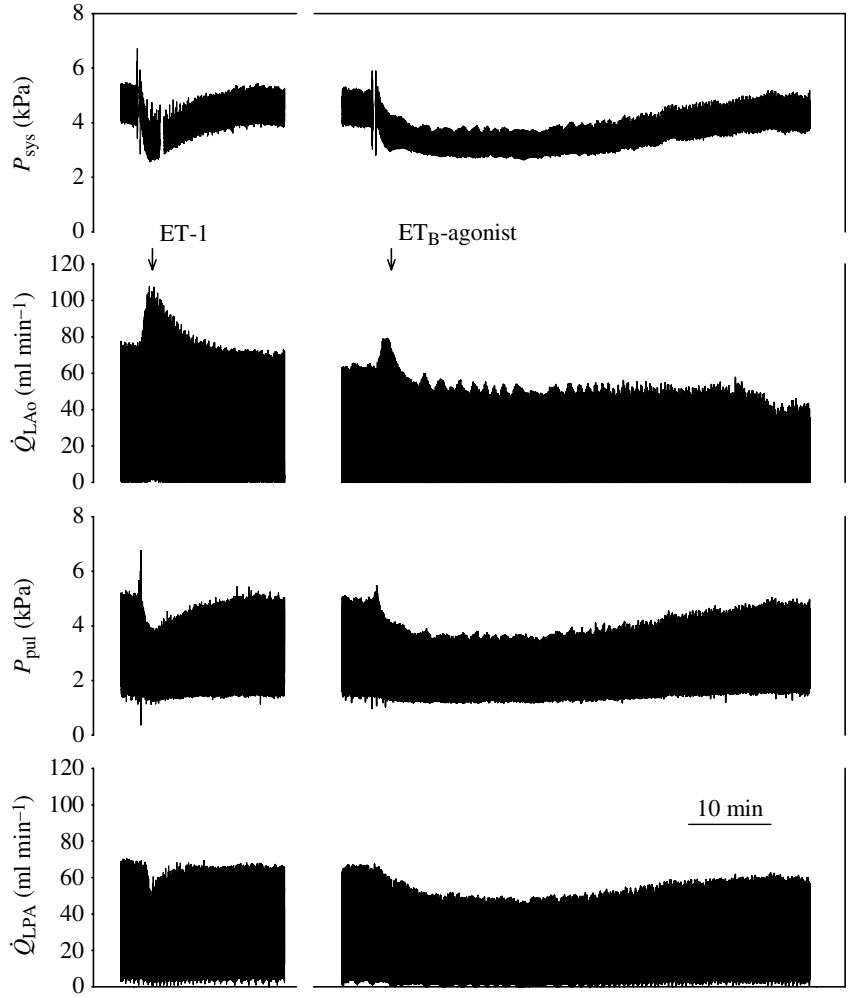

Fig. 1. Original recordings from one turtle (*Trachemys scripta*) showing changes in the recorded variables following injection of ET-1 (120 pmol kg^{-1}) and ET_B-agonist (150 pmol kg^{-1}). Arrows indicate the time of injection. P_{sys}, systemic arterial pressure; $\dot{Q}_{LAo}$, left aortic blood flow; P_{pul}, pulmonary arterial pressure; $\dot{Q}_{LPA}$, left pulmonary blood flow.

catheters were connected to Baxter Edward (model PX600; Irvine, CA, USA) disposable pressure transducers, and the signals were amplified using an in-house-built preamplifier.

For measurements of blood flows, 2S or 2R transit-time ultrasonic blood flow probes (Transonic System, Inc., Ithaca, NY, USA) were placed around the left aortic arch (LAo) and the left pulmonary artery (LPA). Acoustical gel was infused around the blood flow probes to enhance the signal. Both flow probes were connected to a Transonic dual-channel blood flow meter (T206). Signals from the pressure transducers and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at 100 Hz.

Experimental protocols

The study consisted of two separate experimental protocols on different animals. All experiments were carried out at room temperature (20–22°C).

Effects of increased dosages of ET-1 and the effects of ET_B-agonist and ET_A-antagonist (Protocol 1)

Haemodynamic variables were allowed to stabilise over a period of 45 min after instrumentation so that baseline values could be obtained. Then, 1 ml kg^{-1} of isotonic saline (0.9% w/v) containing 0.1% (w/v) albumin was given as a sham infusion to evaluate whether the vehicle for ET-1 had haemodynamic effects. Eight animals then received a series of bolus injections of increasing doses of ET-1 as follows: 0.4, 1.2, 4, 12, 40, 120 and 400 pmol kg^{-1}. To perform a preliminary investigation into the ET-receptor subtypes involved in the haemodynamic changes observed during the dose–response characterisation, we then infused, in the following sequence, the ET_B-agonist BQ-3020 (150 pmol kg^{-1}; 0.15 μmol l^{-1}), ET-1 (120 pmol kg^{-1}; 0.1 μmol l^{-1}), the ET_A-antagonist BQ-610 (0.15 μmol kg^{-1}; 0.15 mmol l^{-1}) and finally ET-1 (120 pmol kg^{-1}) in seven of the eight turtles. All drugs were administered through an arterial catheter in 1 ml kg^{-1} aliquots. Haemodynamic variables were allowed to return to baseline between each injection, which took up to 30 min at the highest dosages, and the ET_A-antagonist was allowed 5 min to distribute and take effect before the subsequent injection of ET-1. A typical Protocol 1 lasted between 4 and 5 h.

Effects of specific inhibition of ET_B-receptors and general block of ET receptors (Protocol 2)

Because the first experimental series revealed a pronounced systemic vasodilatation and similar effects after infusion of the ET_B-agonist, we characterised the effects of ET-1 before and after specific blockade of the ET_B-receptors with the ET_B-antagonist BQ-788 (0.15 μmol kg^{-1}; 0.15 mmol l^{-1}; N=5). This was followed by an additional infusion of ET-1 after administration of the general ET-antagonist tezosentan (15.4 μmol kg^{-1}; 15.4 mmol l^{-1}), which blocks both ET_A- and ET_B-receptors (Clozel et al., 1999).

Tezosentan was obtained as a generous gift from Actelion Pharmaceuticals (Allschwill, Switzerland), whilst all other drugs were purchased from Sigma.

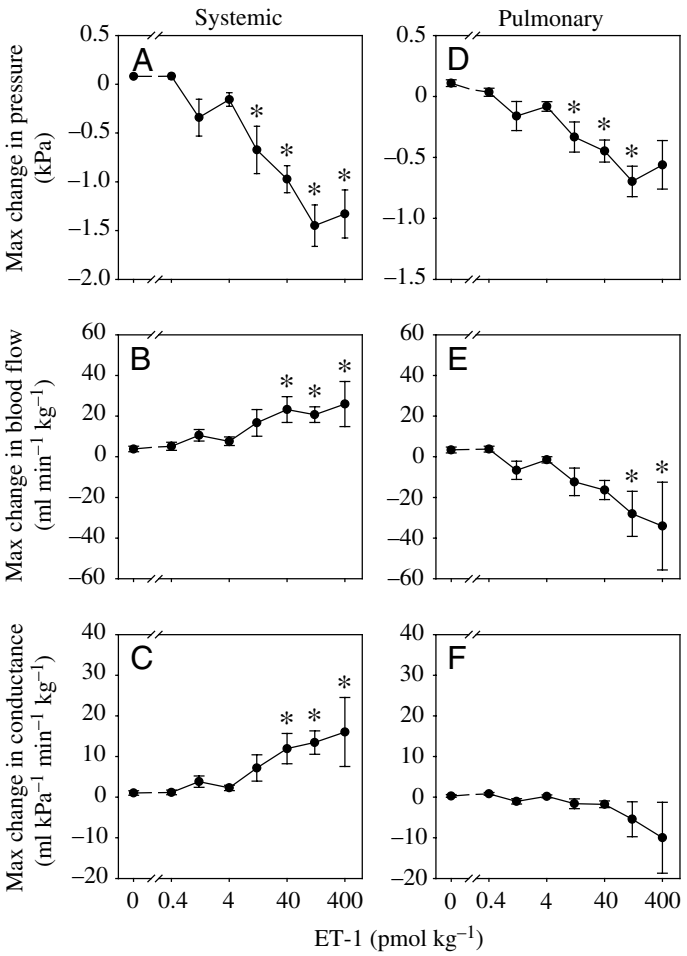

Fig. 2. Effects of bolus intra-arterial injections of ET-1 on the maximum change in mean arterial systemic and pulmonary pressures, (A) P_{sys} and (D) P_{pul}, respectively; systemic and pulmonary blood flows, (B) $\dot{Q}_{sys}$ and (E) $\dot{Q}_{pul}$; systemic and pulmonary vascular conductances, (C) G_{sys} and (F) G_{pul} as a function of the amount of peptide injected. Data points show means ± S.E.M. for eight (systemic variables) and six (pulmonary variables) individual experiments. The asterisks denote a significant difference (P<0.05) from the pre-injection value.

Calculation of blood flows, stroke volume and vascular conductances

Systemic blood flow ($\dot{Q}_{sys}$) was estimated as $2.85\dot{Q}_{LAo}$, and pulmonary blood flow ($\dot{Q}_{pul}$) calculated as $2\dot{Q}_{LPA}$ (Comeau and Hicks, 1994; Wang and Hicks, 1996). Total cardiac output ($\dot{Q}_{tot}$) was calculated as $\dot{Q}_{sys}+\dot{Q}_{pul}$. Heart rate ($f_H$) was calculated from the instantaneous blood flow trace from the LAo, and total stroke volume (V_{Stot}; pulmonary + systemic) was calculated as $\dot{Q}_{tot}/f_H$. When baseline blood flow changes more than baseline blood pressure, which is the case in most *in vivo* situations, conductance provides a better index for comparing vascular tone than resistance (Lautt, 1989; O'Leary, 1991). Systemic and pulmonary conductance (G_{pul} and G_{sys}, respectively) were calculated from mean blood flow and mean blood pressure ($G_{pul}=\dot{Q}_{pul}/P_{pul}$ and $G_{sys}=\dot{Q}_{sys}/P_{sys}$), assuming that central venous blood pressures are negligible.

Table 1. *Haemodynamic effects of ET-1, ET_B-agonist, ET_A-antagonist and ET-1 before and after ET_A-receptor blockade in the freshwater turtle,* Trachemys scripta *(Protocol 1)*

	P_{sys} (kPa)	$\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	G_{sys} (ml kPa^{-1} min^{-1} kg^{-1})	P_{pul} (kPa)	$\dot{Q}_{pul}$ (ml min^{-1} kg^{-1})	G_{pul} (ml kPa^{-1} min^{-1} kg^{-1})	f_H (min^{-1})	$\dot{Q}_{tot}$ (ml min^{-1} kg^{-1})	Vs_{tot} (ml kg^{-1})
Resting	4.1±0.3	34.5±9.0	9.1±2.9	2.8±0.2	69.5±13.8	24.7±5.4	35.7±1.6	96.0±15.7	2.7±0.4
ET-1	2.6±0.3*	55.3±12.3*	22.5±5.5*	2.1±0.3*	41.4±4.4*	19.3±1.4	35.5±2.1	85.9±9.5	2.4±0.3
Resting	4.0±0.4	31.8±7.5	9.1±2.6	2.9±0.3	66.7±12.1	24.0±4.8	36.6±2.7	88.0±11.8	2.6±0.3
ET_B-agonist	3.4±0.3*	48.9±11.1*	17.3±5.5*	2.7±0.2	61.8±9.7	24.3±4.8	36.7±2.8	95.1±7.8	2.8±0.2
Resting	3.5±0.5	22.6±7.5	9.4±5.2	2.7±0.3	55.7±12.4	22.6±5.4	34.1±1.7	70.9±12.3	2.2±0.3
ET-1	2.5±0.5*	32.9±10.8*	17.6±8.1*	2.1±0.4	33.1±5.7	19.0±3.7	34.2±1.7	55.3±5.2	1.8±0.2
Resting	3.5±0.5	21.8±7.7	7.3±3.6	2.6±0.3	54.4±13.5	23.2±4.9	33.4±1.1	68.6±13.7	2.2±0.4
ET_A-antagonist	3.6±0.5	23.8±8.2*	7.7±3.3	2.6±0.4	54.2±14.1	22.5±5.0	33.0±1.2	70.0±14.8	2.2±0.4
Resting	3.6±0.5	23.9±8.4	7.8±3.3	2.5±0.4	53.7±14.6	22.6±5.4	32.8±1.1	69.5±15.3	2.2±0.5
ET-1	2.9±0.5	31.3±9.8*	12.1±4.0*	2.2±0.4	39.0±8.1	20.5±4.1	32.7±1.3	60.7±9.4	2.0±0.3
N	7	7	7	5	6	5	7	6	6

Values are mean ± S.E.M.

P_{sys}, mean systemic arterial pressure; $\dot{Q}_{sys}$, systemic blood flow; G_{sys}, systemic conductance; P_{pul}, mean pulmonary arterial pressure; $\dot{Q}_{pul}$, pulmonary blood flow; G_{pul}, pulmonary conductance; f_H, heart rate; $\dot{Q}_{tot}$, total cardiac output; Vs_{tot}, total stroke volume.

ET-1, endothelin-1 (120 pmol kg^{-1}); ET_B-agonist (150 pmol kg^{-1}); ETA-antagonist (0.15 μmol kg^{-1}).

* denotes a significant difference from resting values (P<0.05).

Data analysis and statistics

All recordings of blood flows and pressures were analysed using AcqKnowledge data analysis software (version 3.7.1.; Biopac). All data presented in the figures were evaluated with a one-way analysis of variance (ANOVA) for repeated measures followed by Tukey or Dunnett *post-hoc* tests. Data presented as percentages, which are not normally distributed, were analysed statistically after an arcsine transformation. The effects of the agonist and antagonists, which are presented as absolute values in the tables, were assessed with paired *t*-tests. Differences were considered statistically significant at a 95% level of confidence (P<0.05), and all data are presented as means ± S.E.M.

Results

Effects of increased dosages of ET-1 and the effects of ET_B-agonist and ET_A-antagonist

The effects of 120 pmol kg^{-1} ET-1 on the recorded variables are depicted in Fig. 1, which also includes the effects of the ET_B-agonist. In both cases, infusion was followed by reductions in systemic and pulmonary blood pressures, attended by a rise in $\dot{Q}_{sys}$ and reciprocal decline in $\dot{Q}_{pul}$. The mean maximum changes to ET-1 infusion are presented in Figs 2, 3, which clearly show that the haemodynamic effects are dose dependent. Injection of ET-1 caused a systemic vasodilatation that was associated with a decrease in P_{sys} and a rise in $\dot{Q}_{sys}$, causing G_{sys} to increase (Fig. 2A–C). There were no effects of ET-1 on G_{pul}, f_H and Vs_{tot} but there was a dose-dependent reduction in $\dot{Q}_{pul}/\dot{Q}_{sys}$ (Figs 2F, 3B–D). The reduction in $\dot{Q}_{pul}/\dot{Q}_{sys}$ at the higher dosages reflects a reversal from a net L–R shunt ($\dot{Q}_{pul}$–$\dot{Q}_{sys}$) to a net R–L shunt (Tables 1, 2).

The effects of subsequent infusion of ET-1, the ET_B-agonist and the ET_A-antagonist are listed in Table 1. The ET_B-agonist elicited haemodynamic changes that were qualitatively similar to those following ET-1, causing a reduction in P_{sys} and an increase in $\dot{Q}_{sys}$ and G_{sys}. Infusion of the ET_A-antagonist resulted in a small increase in $\dot{Q}_{sys}$, but G_{sys} and G_{pul} were not affected (Table 1). Blood flows decreased by 20–30% during Protocol 1 and may reflect progressive deterioration of the experimental preparation, but may also be caused by a decrease in sympathetic tone and changes in central blood volume. This progressive decline in flows did not occur in the shorter experimental Protocol 2.

Effects of specific inhibition of ET_B-receptors and general block of ET receptors

The relative changes in the haemodynamic responses to ET-1 (120 pmol kg^{-1}) before and after administration of the ET_B-antagonist and tezosentan are shown in Fig. 4, and the absolute values are listed in Table 2. The overall responses to ET-1 were similar to those observed in the first experimental protocol, with the exception of the increase in

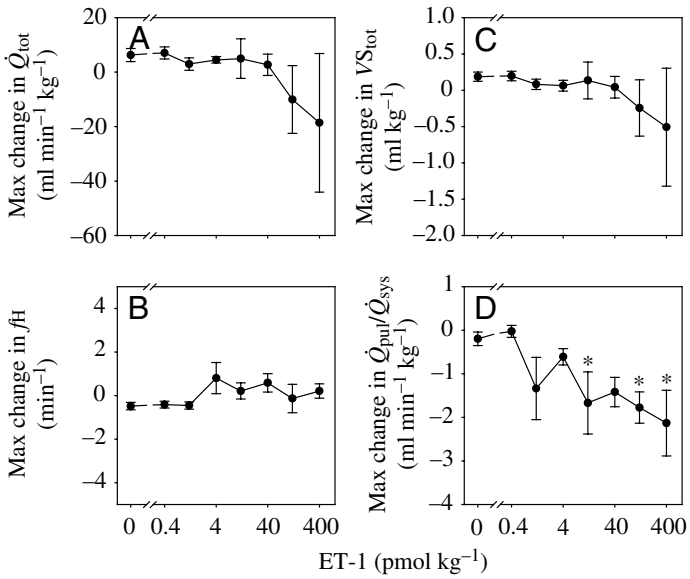

Fig. 3. Effects of bolus intra-arterial injections of ET-1 on the maximum change in (A) total cardiac output ($\dot{Q}_{tot}$), (B) heart rate (f_H), (C) total stroke volume (Vs_{tot}) and (D) $\dot{Q}_{pul}/\dot{Q}_{sys}$ as a function of the amount of peptide injected. Data points show means ± S.E.M. for seven individual experiments. The asterisks denote a significant difference (P<0.05) from the pre-injection value.

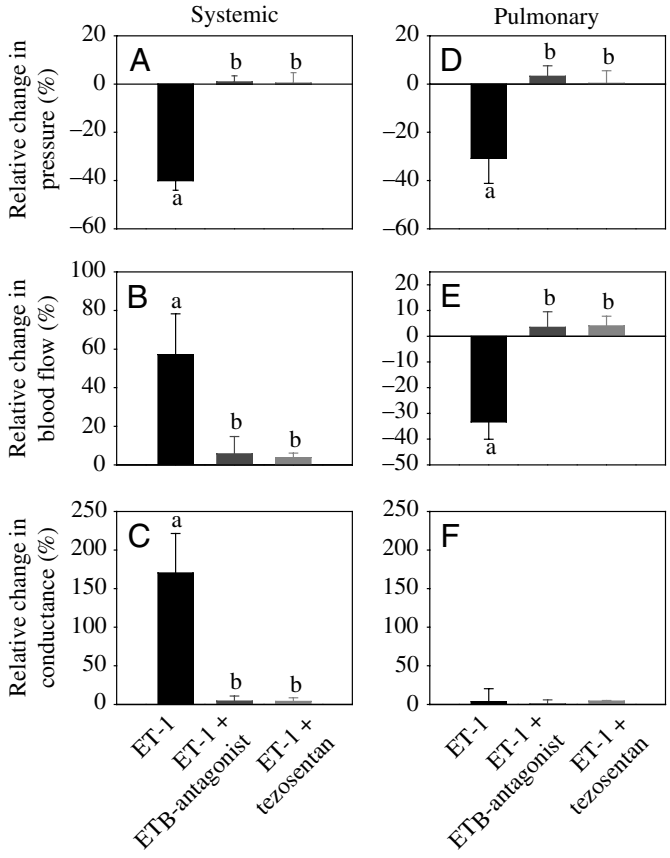

Fig. 4. Effects of bolus injections of ET-1 (120 pmol kg^{-1}) before pharmacological manipulation and after infusion of ET_B-antagonist (0.15 μmol kg^{-1}) and tezosentan (15.4 μmol kg^{-1}) on relative changes in haemodynamic variables. Data points show means ± S.E.M. for five individual experiments. Bars marked with different letters are significantly different from each other (P<0.05).

$\dot{Q}_{sys}$ being non-significant (P=0.052). Infusion of the ET_B-antagonist had no effects on vascular conductances but caused a rise in P_{sys}, $\dot{Q}_{pul}$ and $\dot{Q}_{tot}$ (Table 2). All effects of ET-1, however, were abolished after ET_B-receptor blockade (Fig. 4). The subsequent treatment with the general ET-receptor antagonist tezosentan did not produce effects that differed from the treatment with ET_B-antagonist, and the blockade of ET-1 responses persisted.

Discussion

The turtles exhibited a marked and long-lasting systemic vasodilatation, expressed as a decrease in P_{sys} and a rise in $\dot{Q}_{sys}$, upon infusion of ET-1. There was no secondary vasoconstriction. Heart rate did not increase in response to lowering of systemic blood pressure, which indicates that the baroreflex is suppressed by the pentobarbital anaesthesia. Indeed, using the same experimental preparation, we have shown that the heart of turtles does not respond to large changes in blood pressure following infusion of sodium nitroprusside and phenylephrine (M. Zaar and T. Wang, unpublished), whereas fully recovered turtles do exhibit normal barostatic responses (Millard and Moalli, 1980).

The lack of a secondary pressor response differs from mammals. In mammals, ET-1 causes an initial, but transient, vasodilatation that usually lasts for less than 1 min, which is followed by a prolonged and dose-dependent vasoconstriction that reaches a plateau 5–10 min after infusion and often persists for more than 30 min (Mateo and Artiñano, 1997). This response occurs in both anaesthetised and awake rats (Knuepfer et al., 1989), so it is unlikely that the differences between mammals and reptiles are due to the influence of anaesthesia. Trout and alligators also exhibit a biphasic response (Olson et al., 1991; Wang et al., 2000; Platzack et al., 2002), but the initial vasodilatation in alligators is more pronounced and longer in duration than that reported for mammals, and the subsequent systemic vasoconstriction is only present after large dosages of ET-1 (Platzack et al., 2002). The lack of a vasoconstriction in turtles may seem surprising, as Poder et al. (1991) showed that rings of the LAo and the pulmonary artery from *Trachemys scripta* constrict *in vitro* when exposed to ET-1. Similar responses were obtained on aortic rings from catfish and frogs (Poder et al., 1991). It is possible that the endothelium was damaged during these ring preparations, which would reduce the initial dilatation resulting from endothelial ET_B-receptor stimulation, or that the constrictor response is confined to these large vessels, which do not contribute significantly to overall vascular resistance.

An abundant occurrence of ET_B-receptors in endothelial cells has been localised in various tissues of the lizard *Podarcis sicula* (De Falco et al., 2002), but our study is the first to investigate the

Table 2. *Haemodynamic effects of ET-1, ET_B-antagonist, tezosentan and ET-1 before and after ET_B- and general ET-receptor blockade in the freshwater turtle,* Trachemys scripta *(Protocol 2)*

	P_{sys} (kPa)	$\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	G_{sys} (ml kPa^{-1} min^{-1} kg^{-1})	P_{pul} (kPa)	$\dot{Q}_{pul}$ (ml min^{-1} kg^{-1})	G_{pul} (ml kPa^{-1} min^{-1} kg^{-1})	f_H (min^{-1})	$\dot{Q}_{tot}$ (ml min^{-1} kg^{-1})	Vs_{tot} (ml kg^{-1})
Resting	4.0±0.4	42.7±10.4	10.7±2.3	2.9±0.3	84.7±23.7	26.3±9.9	35.9±5.6	128.3±36.9	4.5±1.6
ET-1	2.5±0.2*	65.0±15.8	25.2±5.3*	2.0±0.4*	59.1±21.8*	26.1±9.6	39.7±3.0	126.7±40.7	3.3±0.9
Resting	4.1±0.4	33.1±7.6	8.3±1.5	2.8±0.4	79.7±21.3	26.5±9.7	31.5±6.4	114.6±29.6	5.3±2.1
ET_B-antagonist	4.4±0.4*	35.1±7.1	7.9±1.3	3.0±0.4	89.0±23.5*	28.2±9.7	31.5±5.5	126.0±31.7*	5.1±1.6
Resting	4.5±0.4	35.0±7.0	7.8±1.2	2.9±0.4	86.8±22.4	27.7±9.4	31.1±5.4	123.7±30.4	5.1±1.6
ET-1	4.4±0.4	37.6±8.3	8.6±1.5	3.0±0.3	89.5±24.5	27.6±9.1	31.4±5.4	129.1±33.9	5.2±1.6
Resting	4.7±0.2	27.7±7.0	6.0±1.5	2.9±0.3	84.3±17.1	27.1±7.7	29.6±6.3	114.8±21.4	6.3±2.7
Tezosentan	4.5±0.2	30.5±7.6	7.0±2.1	3.0±0.3	79.0±5.8	25.6±4.6	32.4±6.4	112.3±13.4	5.3±2.1
Resting	4.5±0.2	30.1±7.5	6.9±2.0	3.0±0.2	79.0±6.1	25.7±4.7	32.2±6.3	111.9±13.6	5.3±2.1
ET-1	4.5±0.1	31.2±7.2	6.9±1.7	3.0±0.2	82.6±8.3	26.8±4.9	31.9±6.1	116.4±16.1	5.3±2.0
N	5	5	5	3	4	3	5	4	4

Values are mean ± S.E.M.

P_{sys}, mean systemic arterial pressure; $\dot{Q}_{sys}$, systemic blood flow; G_{sys}, systemic conductance; P_{pul}, mean pulmonary arterial pressure; $\dot{Q}_{pul}$, pulmonary blood flow; G_{pul}, pulmonary conductance; f_H, heart rate; $\dot{Q}_{tot}$, total cardiac output; Vs_{tot}, total stroke volume.

ET-1, endothelin-1 (120 pmol kg^{-1}); ET_B-antagonist (0.15 μmol kg^{-1}); tezosentan (15.4 μmol kg^{-1}).

* denotes a significant difference from resting values (P<0.05).

haemodynamic role of the different ET-receptors in a non-mammalian tetrapod. Two endothelin receptors have been cloned and pharmacologically characterised in mammals (Sokolovsky, 1995). The ET_A-receptor has high affinity for ET-1 and ET-2 but low affinity for ET-3, while the ET_B-receptor has similar affinities for all three isoforms. In *Xenopus*, an ET_C-receptor has been cloned from heart and lungs, which is pharmacologically similar to the mammalian ET_A-receptor, with the exception of being insensitive to the ET_A-antagonist BQ-123 (Kumar et al., 1994). Stimulation of ET_A-receptors, located within the smooth muscle, generally causes vasoconstriction, and stimulation of ET_B-receptors located in the endothelium leads to vasodilatation (Mateo and Artiñano, 1997; Masaki, 2004). However, ET_B-receptors are also located in smooth muscle, and stimulation of these receptors can lead to constriction (Mateo and Artiñano, 1997). This seems to be the case in isolated aortic rings from sharks, where stimulation of ET_B-receptors leads to constriction in the presence and absence of an intact endothelium (Evans et al., 1996).

In turtles, the ET_B-agonist BQ-3020 caused a pronounced vasodilatation that qualitatively resembled that elicited by ET-1, and the entire response to ET-1 could be completely abolished by the ET_B-antagonist BQ-788. Subsequent treatment with the general ET receptor antagonist tezosentan did not produce effects that differed from those of the ET_B-antagonist. These observations clearly indicate that ET_B-receptors mediate the systemic vasodilatation in response to ET-1 in *Trachemys*. In mammals, the vasodilatation following ET_B-receptor stimulation is often mediated by increased NO liberation (Moritoki et al., 1993). Turtles exhibit the same systemic vasodilatation in response to exogenously administrated NO as other vertebrates (Crossley et al., 2000), but the possible role of NO in the dilation following ET-1 was not investigated in our study. However, the initial systemic vasodilatation caused by ET-1 was not affected by NOS inhibition in alligators, suggesting that ET_B-receptors activate other endothelium-derived relaxing factors, such as prostaglandins or leukotrienes (Platzack et al., 2002).

There are no good and easily available ET_A-agonists, so the putative involvement of ET_A-receptors was only investigated by blockade of ET_A-receptors. While our data do show an attenuated cardiovascular response to ET-1 after treatment with the ET_A-antagonist, it is likely that this reduction in the response reflects tachyphylaxis, as there seems to be a progressive reduction in the responsiveness throughout the experimental protocol (Table 1). Also, given that ET_A-receptor stimulation normally induces a pressor response in fish and mammals, we would have expected ET_A-receptor blockade to enhance the vasodilatation in response to ET-1. Thus, it seems unlikely that ET_A-receptors are important in the systemic vasculature of turtles.

There were only small direct effects of the various ET-antagonists used in our study (Tables 1, 2). This indicates that endothelin contributes very little to the maintenance of

the systemic vascular tone in anaesthetised turtles but does not rule out the possibility that endothelin may have more pronounced effects in fully recovered animals under various conditions. Because turtles exhibit pronounced cardiovascular changes during the intermittent breathing pattern (Shelton and Burggren, 1979; White et al., 1989; Wang and Hicks, 1996), it is convenient to study the roles of various local factors in anaesthetised animals where autonomic regulation of the cardiovascular system is reduced (Crossley et al., 1998).

The pulmonary vascular conductance of turtles was not affected by ET-1 or any of the ET agonists and antagonists. Thus, the reduction in $\dot{Q}_{pul}$ that follows the infusion of ET-1 reflects the systemic vasodilatation that directs blood flows from the undivided ventricle towards the systemic circulation. Therefore, ET-1 induced a significant rise in the right-to-left cardiac shunt (Fig. 3D). The lack of an effect of ET-1 is consistent with the alligator, where only very high dosages affected the pulmonary circulation (Platzack et al., 2002). However, ET_B-receptors are abundant in the endothelium of the pulmonary arteries in the lizard *Podarcis sicula* (De Falco et al., 2002), and isolated rings of the pulmonary artery from turtles constrict in response to ET-1 (Poder et al., 1991). In the mammalian lung, endothelin normally causes vasoconstriction and has been implicated in pulmonary hypertension as well as hypoxic pulmonary vasoconstriction (Goldie et al., 1996a,b; Michael and Markewitz, 1996). Interestingly, in fish gills, the functional analogue to the lungs of tetrapods, ET-1 leads to a marked vasoconstriction that seems to be caused by contraction of the pillar cells in the gill lamellae (Sundin and Nilsson, 1998; Stensløkken et al., 1999). The lack of effects of ET-1 in the turtle lung is consistent with the very small effects of various regulatory peptides and NO in the pulmonary circulation of most reptiles studied so far (Crossley et al., 2000; Platzack et al., 2002; Galli et al., 2005a,b; Skovgaard et al., 2005).

In conclusion, the present study reveals a potentially important role for ET-1 in regulating systemic vascular tone in turtles. However, unlike mammals, but consistent with alligators, the primary role of this endothelium-derived peptide appears to be vasodilatation. In turtles, this response is mediated through stimulation of ET_B-receptors, whereas ET_A-receptors are of minor importance.

This study was supported by the Danish Research Council, the NOVO Foundation and the National Science Foundation (IBN 01-10322 to D.C.J.).

References

Alonso, D. and Radomski, M. W. (2003). The nitric oxide-endothelin-1 connection. *Heart Fail. Rev.* **8**, 107-115.

Clozel, M., Ramuz, H., Clozel, J.-P., Breu, V., Hess, P., Löffler, B.-M., Coassolo, P. and Roux, S. (1999). Pharmacology of tezosentan, new endothelial receptor antagonist designed for parenteral use. *J. Pharm. Exp. Ther*. **290**, 840-846.

Comeau, S. and Hicks, J. W. (1994). Regulation of central vascular blood flow in the turtle. *Am. J. Physiol.* **267**, R569-R578.

Crossley, D. A., Altimiras, J. and Wang, T. (1998). Hypoxia elicits pulmonary vasoconstriction in anaesthetized turtles. *J. Exp. Biol.* **201**, 3367-3375.

Crossley, D. A., Wang, T. and Altimiras, J. (2000). Role of nitric oxide in the systemic and pulmonary circulation of anesthetized turtles (*Trachemys scripta*). *J. Exp. Zool.* **286**, 683-689.

De Falco, M., Laforgia, V., Fedele, V., Russo, T., De Luca, L., Cottone, G., Varano, L. and De Luca, A. (2002). Endothelin-B (ET_B) receptor distribution in tissues of the lizard *Podarcis sicula*. *Cell Tissue Res*. **309**, 381-386.

Evans, D. H., Gunderson, M. and Cegelis, C. (1996). ET(B)-type receptors mediate endothelin stimulated contraction in the aortic vascular smooth muscle of the spiny dogfish shark, *Squalus acanthias*. *J. Comp. Physiol. B* **165**, 659-664.

Galli, G. L. J., Skovgaard, N., Abe, A. S., Taylor, E. W., Conlon, J. M. and Wang, T. (2005a). Cardiovascular actions of rattlesnake bradykinin ([Val^1Thr^6]bradykinin) in the anesthetized South American rattlesnake, *Crotalus durissus terrificus*. *Am. J. Physiol.* **288**, 456-465.

Galli, G., Skovgaard, N., Abe, A. S., Taylor, E. W. and Wang, T. (2005b). The role of nitric oxide in the regulation of the systemic and the pulmonary vasculature of the rattlesnake, *Crotalus durissus terrificus*. *J. Comp. Physiol. B* **175**, 201-208.

Goldie, R. G., D'Aprile, A. C., Self, G. J., Rigby, P. J. and Henry, P. J. (1996a). The distribution and density of receptor subtypes for endothelin-1 in peripheral lung of the rat, guinea-pig and pig. *Br. J. Pharmacol*. **117**, 729-735.

Goldie, R. G., Knott, P. G., Carr, M. J., Hay, D. W. P. and Henry, P. J. (1996b). The endothelins in the pulmonary system. *Pulm. Pharmacol*. **9**, 69-93.

Hicks, J. W. (1998). Cardiac shunting in reptiles: mechanisms, regulation and physiological function. In *Biology of the Reptilia. The Visceral Organs*, vol. G (ed. C. Gans and A. S. Gaunt), pp. 425-483. Ithaca, NY: Society for the Study of Amphibians and Reptiles.

Hoagland, T. M., Weaver, L., Jr, Conlon, J. M., Wang, Y. and Olson, K. R. (2000). Effects of endothelin-1 and homologous trout endothelin on cardiovascular function in rainbow trout. *Am. J. Physiol*. **278**, R460-R468.

Knuepfer, M. M., Han, S. P., Trapani, A. J., Fok, K. F. and Westfall, T. C. (1989). Regional hemodynamic and baroreflex effects of endothelin in rats. *Am. J. Physiol.* **257**, H918-H926.

Kumar, C., Mwangi, V., Nuthulaganti, P., Wu, H. L., Pullen, M., Brun, K., Aiyar, H., Morris, R. A., Naugthon, R. and Nambi, P. (1994). Cloning and characterization of a novel endothelin receptor from *Xenopus* heart. *J. Biol. Chem*. **269**, 13414-13420.

Landan, G., Bdolah, A., Wollberg, Z., Kochva, E. and Grauer, D. (1991). Evolution of the sarafotoin/endothelin superfamily of proteins. *Toxicon* **29**, 237-244.

Lautt, W. W. (1989). Resistance or conductance for expression of arterial vascular tone. *Microvasc. Res*. **37**, 230-236.

Masaki, T. (2004). Historical review: endothelin. *Trends Pharmacol. Sci*. **25**, 219-224.

Mateo, A. O. and de Artiñano, A. A. (1997). Highlights on endothelin: a review. *Pharmacol. Res.* **36**, 339-351.

Michael, J. R. and Markewitz, B. A. (1996). Endothelins and the lung. *Am. J. Respir. Crit. Care Med*. **154**, 555-581.

Millard, R. W. and Moalli, R. (1980). Baroreflex sensitivity in an amphibian, *Rana catesbeiana*, and a reptilian, *Pseudemys scripta elegans*. *J. Exp. Zool*. **213**, 283-288.

Miyauchi, T. and Masaki, T. (1999). Pathophysiology of endothelin in the cardiovascular system. *Annu. Rev. Physiol.* **61**, 391-415.

Moritoki, H., Miyano, H., Takeuchi, S., Yamaguchi, M., Hisayama, T. and Kondoh, W. (1993). Endothelin-3-induced relaxation of rat thoracic aorta: a role for nitric oxide formation. *Br. J. Pharmacol*. **108**, 1123-1130.

O'Leary, D. S. (1991). Regional vascular resistance *vs*. conductance: which index for baroreflex responses? *Am. J. Physiol.* **260**, H632-H637.

Olson, K. R., Duff, D. W., Farrell, A. P., Keen, J., Kellogg, M. D., Kullman, D. and Villa, J. (1991). Cardiovascular effects of endothelin in trout. *Am. J. Physiol*. **29**, H1214-H1223.

Page, R. D. M. (2000). Extracting species trees from complex gene trees: reconciled trees and vertebrate phylogeny. *Mol. Phylogenet. Evol*. **14**, 89-106.

Platzack, B., Wang, Y., Crossley, D., Lance, V., Hicks, J. W. and Conlon, J. M. (2002). Characterization and cardiovascular actions of endothelin-1 and endothelin-3 from the American alligator. *Am. J. Physiol*. **282**, R594-R602.

Poder, T. C., Silderberg, S. D. and Rampe, D. (1991). Contraction of reptile,

amphibian, and fish blood vessels by endothelin-1. *Can J. Physiol. Pharmacol*. **69**, 215-217.

Shelton, G. and Burggren, W. W. (1976). Cardiovascular dynamics of chelonian during apnea and lung ventilation. *J. Exp. Biol.* **64**, 323-343.

Skovgaard, N., Galli, G., Taylor, E. W., Conlon, J. M. and Wang, T. (2005). Hemodynamic effects of python neuropeptide γ in the anesthetized python, *Python regius*. *Regul. Peptides* **128**, 15-26.

Sokolovsky, M. (1995). Endothelin receptor subtypes and their role in transmembrane signalling mechanisms. *Pharmacol. Therapeut.* **68**, 435-471.

Stensløkken, K.-O., Sundin, L. and Nilsson, G. E. (1999). Cardiovascular and gill microcirculatory effects of endothelin-1 in Atlantic cod: evidence for pillar cell contraction. *J. Exp. Biol.* **202**, 1151-1157.

Sundin, L. and Nilsson, G. E. (1998). Endothelin redistributes blood flow through the lamellae of rainbow trout gills. *J. Comp. Physiol. B* **168**, 619-623.

Wang, T. and Hicks, J. W. (1996). Cardiorespiratory synchrony in turtles. *J. Exp. Biol.* **199**, 1791-1800.

Wang, Y., Olson, K. R., Smith, M. P., Russell, M. J. and Conlon, J. M. (1999). Purification, structural characterization, and myotropic activity of endothelin from trout, *Oncorhynchus mykiss*. *Am. J. Physiol*. **277**, R1605-R1611.

Wang, Y., Remy-Jouet, I., Delarue, C., Letourneau, M., Fournier, A., Vaudry, H. and Conlon, J. M. (2000). Structural characterization and effects on corticosteroid secretion of endothelin-1 and endothelin-3 from the frog, *Rana ridibunda*. *J. Mol. Endocrinol*. **24**, 285-293.

White, F. N., Hicks, J. W. and Ishimatsu, A. (1989). Relationship between respiratory state and intracardiac shunts in reptiles. *Am. J. Physiol*. **256**, R240-R247.

Yanagisawa, M. and Masaki, T. (1989). Endothelin, a novel endothelium-derived peptide. *Biochem. Pharmacol*. **38**, 1877-1883.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial-cells. *Nature* **332**, 411-415.