The role of nitric oxide in regulation of the cardiovascular system in reptiles

Article *in* Comparative Biochemistry and Physiology - Part A Molecular & Integrative Physiology · November 2005

DOI: 10.1016/j.cbpb.2005.05.049 · Source: PubMed

CITATIONS
27

READS
65

5 authors, including:

Gina Galli
The University of Manchester
70 PUBLICATIONS **1,072** CITATIONS
SEE PROFILE

Augusto S Abe
São Paulo State University
201 PUBLICATIONS **4,539** CITATIONS
SEE PROFILE

Edwin Taylor
University of Birmingham
227 PUBLICATIONS **7,969** CITATIONS
SEE PROFILE

Tobias Wang
Aarhus University
509 PUBLICATIONS **9,901** CITATIONS
SEE PROFILE

ELSEVIER

Comparative Biochemistry and Physiology, Part A 142 (2005) 205 – 214

CBP

www.elsevier.com/locate/cbpa

The role of nitric oxide in regulation of the cardiovascular system in reptiles☆

Nini Skovgaard [a,b,*], Gina Galli [a,b,c], Augusto Abe [a], Edwin W. Taylor [a,c], Tobias Wang [a,b]

[a]Departamento de Zoologia, Centro de Aguicultura, UNESP, Caixa Postal 199, 13506-907 Rio Claro, Brazil
[b]Department of Zoophysiology, Aarhus University, Building 131, 8000 Aarhus C, Denmark
[c]School of Biosciences, University of Birmingham, Edgbaston, Birmingham B19 2TT, England

Received 22 February 2005; received in revised form 20 May 2005; accepted 21 May 2005
Available online 27 June 2005

Abstract

The roles that nitric oxide (NO) plays in the cardiovascular system of reptiles are reviewed, with particular emphasis on its effects on central vascular blood flows in the systemic and pulmonary circulations. New data is presented that describes the effects on hemodynamic variables in varanid lizards of exogenously administered NO via the nitric oxide donor sodium nitroprusside (SNP) and inhibition of nitric oxide synthase (NOS) by L-nitroarginine methyl ester (L-NAME). Furthermore, preliminary data on the effects of SNP on hemodynamic variables in the tegu lizard are presented. The findings are compared with previously published data from our laboratory on three other species of reptiles: pythons (Skovgaard, N., Galli, G., Taylor, E.W., Conlon, J.M., Wang, T., 2005. Hemodynamic effects of python neuropeptide γ in the anesthetized python, Python regius. Regul. Pept. 18, 15–26), rattlesnakes (Galli, G., Skovgaard, N., Abe, A.S., Taylor, E.W., Wang, T., 2005. The role of nitric oxide in the regulation of the systemic and the pulmonary vasculature of the rattlesnake, Crotalus durissus terrificus. J. Comp. Physiol. 175B, 201–208) and turtles (Crossley, D.A., Wang, T., Altimiras, J., 2000. Role of nitric oxide in the systemic and pulmonary circulation of anesthetized turtles (Trachemys scripta). J. Exp. Zool. 286, 683–689). These five species of reptiles possess different combinations of division of the heart and structural complexity of the lungs. Comparison of their responses to NO donors and NOS inhibitors may reveal whether the potential contribution of NO to vascular tone correlates with pulmonary complexity and/or with blood pressure. All existing studies on reptiles have clearly established a potential role for NO in regulating vascular tone in the systemic circulation and NO may be important for maintaining basal systemic vascular tone in varanid lizards, pythons and turtles, through a continuous release of NO. In contrast, the pulmonary circulation is less responsive to NO donors or NOS inhibitors, and it was only in pythons and varanid lizards that the lungs responded to SNP. Both species have a functionally separated heart, so it is possible that NO may exert a larger role in species with low pulmonary blood pressures, irrespective of lung complexity.

Keywords: Nitric oxide; L-NAME; Reptiles; *Varanus*; *Trachemys*; *Python*; *Crotalus*; Cardiovascular; Local regulation; Blood pressure; Blood flow

1. Introduction

The evolution of endothermy was associated with a large rise in resting and maximal rates of oxygen consumption (e.g. Bennett and Ruben, 1979). The transition required higher resting and maximal heart rates to match the increased demands of oxygen delivery, and a rise in blood pressure to secure an adequate perfusion pressure for the higher capillary density (Burggren et al., 1997). In the systemic circulation, the higher capillary density reduced the diffusive distances for respiratory gases and nutrients, but the increased vascular complexity also increased possibilities for inhomogeneities between perfusion and metabolism (Piiper and Scheid, 1991; Piiper, 2000). In the pulmonary circulation, higher structural complexity allowed for smaller

☆ From: ***Nitric Oxide: Comparative aspects of respiratory and cardiovascular homeostasis***, an SEB symposium, organised by Prof. Bruno Tota (University of Calabria, Italy) and Prof. Tobias Wang (University of Aarhus, Denmark)—Capri, Italy, September, 2004.

* Corresponding author. Department of Zoophysiology, Aarhus University, Building 131, 8000 Aarhus C, Denmark.
E-mail address: nini.jensen@biology.au.dk (N. Skovgaard).

1095-6433/$ - see front matter. © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2005.05.049

gas exchange units and increased surface area, which increased the pulmonary diffusive capacity for O_2. However, the higher complexity probably increased the possibilities for local mismatching of perfusion and ventilation (Piiper, 1992; Powell and Hopkins, 2004).

In both the systemic and pulmonary circulations, inhomogeneities between perfusion and metabolism and perfusion and ventilation, respectively, exert significant constraints on gas exchange (Piiper and Scheid, 1991; Piiper, 1992, 2000). However, this problem is partially overcome by the local release of metabolites and regulatory factors, (e.g. catecholamines, nitric oxide and various regulatory peptides) that match oxygen delivery to metabolic demands by affecting vasomotion in local vascular beds (Burnstock, 1990; Alberola et al., 1994; Barnes and Liu, 1995; Delp and O'Leary, 2004). These effects have mostly been studied in mammals, but are likely to prevail in all vertebrates.

Reptiles represent a phylogenetically important group because endothermic birds and mammals evolved independently from reptilian ancestors (Page, 2000), and the different taxonomic groups of reptiles vary substantially with regards to heart morphology and lung structure (Perry, 1989; Hicks, 1998; Wang et al., 1998). In non-crocodilian reptiles, with the exception of pythons and varanid lizards (Burggren and Johansen, 1982; Wang et al., 2003), the ventricle is anatomically and functionally undivided, so blood pressures are equal in the systemic and pulmonary circulations (e.g. Hicks, 1998). In these species, blood flow distribution between the two circulations is primarily determined by their relative vascular resistances (Hicks, 1998; Crossley et al., 1998). Varanid lizards and pythons, however, have a functionally divided ventricle, which allows for high, mammalian-like systemic blood pressures, while pulmonary blood pressures are low (Burggren and Johansen, 1982; Wang et al., 2003). In most snakes and many lizards, the lung structure is simple, whereas the lungs in turtles, varanid lizards and crocodilians are more complex and multi-chambered (Perry, 1989).

It is likely that, during the transition from the structurally simple lungs and undivided hearts of reptiles, to the complex lungs and divided hearts of mammals, local and humoral regulation of the systemic and pulmonary circulations have become increasingly more important. This general hypothesis, illustrated as a matrix of selected species in Fig. 1, can be evaluated by studying the local regulation of the cardiovascular system in ectothermic species with varying cardiac and pulmonary morphologies. Compared to mammals, the role of humoral and local factors in

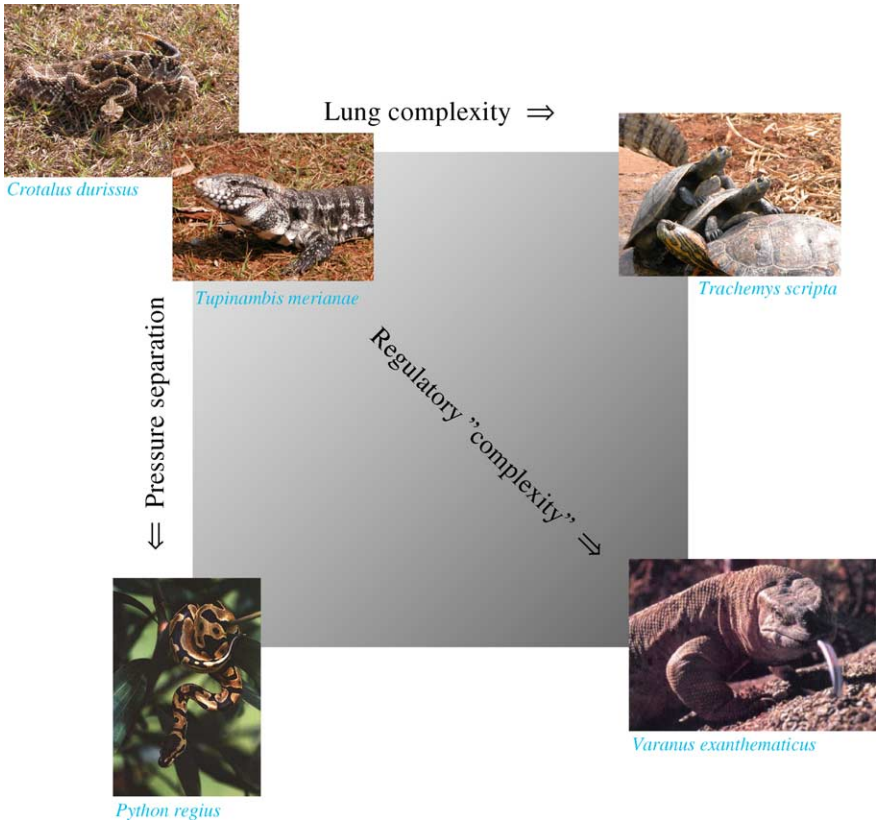

Fig. 1. Different combinations of cardiac division and structural lung complexity in the five studied species: rattlesnake, *Crotalus durissus* (Galli et al., 2005b); turtle, *Trachemys scripta* (Crossley et al., 2000); varanid lizard, *Varanus exanthematicus*; python, *Python regius* (Skovgaard et al., 2005); tegu lizard, *Tupinambis merianae* and the proposed correlation between vascular complexity and an increased role for local regulation including NO control.

cardiovascular control in reptiles remains largely unknown. Recent studies, nevertheless, indicate that the pulmonary circulation is much less responsive to humoral and local factors than the systemic circulation (Conlon et al., 1990; Comeau et al., 1992; Crossley et al., 2000; Platzack et al., 2002; Galli et al., 2005a; Skovgaard et al., 2005).

In mammals, nitric oxide (NO) is a potent vasodilator of the cardiovascular system and NO exerts a crucial role in the regulation of vascular tone (Furchgott and Zawadski, 1980; Palmer et al., 1987; for review see Moncada et al., 1991; Cai and Harrison, 2000). Thus, continuous release of NO from the endothelium maintains a direct dilatory tone, while the vasodilatory action of various vasoactive substances, such as acetylcholine, bradykinin and substance P, is, at least in some cases, mediated through a release of NO (Moncada et al., 1991). NO is synthesized from L-arginine by nitric oxide synthase (NOS) that occurs in three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) (Förstermann et al., 1994). Formed in the endothelium or neurons, NO diffuses into the adjacent smooth muscle cells where it causes relaxation. Both endothelium-derived NO and NO formed in neurons seem to regulate vascular tone in reptiles (Miller and Vanhoutte, 1986; Knight and Burnstock, 1993; Axelsson et al., 2001).

Here we review the cardiovascular effects of NO with particular emphasis on the overall role for regulation of central vascular blood flow in the systemic and pulmonary circulations of reptiles. We present data on the effect of exogenously administrated NO (via the nitric oxide donor SNP) and inhibition of NOS (by the non-specific nitric oxide synthase inhibitor L-NAME) on the systemic and pulmonary vasculature of the varanid lizard (*Varanus exanthematicus*), and preliminary data on the tegu lizard (*Tupinambis merianae*). The findings are compared with previously published data from our laboratory on three other species of reptiles: the python (Skovgaard et al., 2005), the rattlesnake (Galli et al., 2005b) and the turtle (Crossley et al., 2000). These five species of reptiles possess different combinations of cardiac division and structural complexity of the lungs, so by comparing their responses to NO donors and NOS inhibitors, we aim to investigate the hypothesis that nitric oxide exerts a stronger role in species with higher vascular complexity.

2. Materials and methods

2.1. Experimental animals

Experiments were performed on six varanid lizards (*V. exanthematicus*), six pythons (*Python regius*), five turtles (*Trachemys scripta*), and six rattlesnakes (*Crotalus durissus*). In addition, we present observations on one tegu lizard (*T. merianae*). Varanid lizards (mass range 330–990 g) and pythons (550–900 g) were obtained from Danish commercial suppliers, turtles (472–1600 g) were obtained from Lemberger Inc. (Oshokosh, WI, USA) and the tegu lizard (3000 g) was captive-bred at UNESP, Rio Claro (Sao Paulo, Brazil). All animals were freighted to the Department of Zoophysiology, University of Aarhus (Denmark) where experiments were carried out. Rattlesnakes (500–1500 g) were obtained from the Butantan Institute, Sao Paulo, and transported to UNESP, where experiments were conducted. Food was withheld for at least 3 days prior to experimentation and all animals appeared healthy. Experiments were carried out according to Danish and Brazilian Federal Regulations.

2.2. Anesthesia

Pythons, turtles and rattlesnakes were anaesthetized with an intramuscular injection of pentobarbital (Mebumal; turtles, 50 mg kg^{-1}; pythons and rattlesnakes, 30 mg kg^{-1}). All reflexes disappeared within 15–45 min. Due to their low tolerance of hypoxia lizards were rapidly anaesthetized by inhalation of isoflurane vapor (Isofluran, Baxter, Denmark) by placing a plastic bag over their head. All animals were tracheostomized for artificial ventilation using a Harvard Apparatus mechanical ventilator at 15–20 breaths min^{-1} and a tidal volume of 50–100 mL kg^{-1} with 3% CO_2 (balance air). During the initial stages of surgery, the lizards were ventilated with room air containing 1% isoflurane, and when the first cannulation was finished an intra-arterial injection of pentobarbital (Mebumal; 50 mg kg^{-1}) was given, and the isoflurane was turned off.

2.3. Surgery and instrumentation

2.3.1. Varanid lizards

The central vascular blood vessels were accessed through a 5 cm incision in the sternum. For measurement of systemic arterial blood pressure (P_{sys}), the left carotid artery was occlusively cannulated with a PE50 catheter containing heparinized saline. Pulmonary arterial blood pressure (P_{pul}) was measured using a catheter (PE50) inserted into a branch of the left pulmonary artery. For measurements of blood flows, 2S or 2R transit-time ultrasonic blood flow probes (Transonic System, Inc., NY, USA) were placed around the left pulmonary arch and the left aortic arch.

2.3.2. Pythons

A 5 cm ventral incision was made cranial to the heart and a PE50 catheter was advanced into the right aortic arch through the vertebral artery. An additional incision was made immediately above the heart for insertion of a catheter in the left pulmonary artery. The left pulmonary artery, which is much smaller than the right pulmonary artery and carries less than a quarter of the total pulmonary blood flow (unpublished observations by the authors), was occlusively cannulated with a PE50 catheter. Blood flow probes were

placed around the left aortic arch and the right pulmonary artery. (For additional descriptions, see Skovgaard et al., 2005.)

2.3.3. Turtles

Using a bone saw, a 5×5 cm piece of the plastron was removed to expose the central blood vessels. The left carotid artery was occlusively cannulated with a PE90 catheter, and the tip of the catheter forwarded into the right aortic arch. The common pulmonary artery was non-occlusively cannulated using the Seldinger technique (White et al., 1989). Blood flows were measured in the left aortic arch and left pulmonary artery. (For additional descriptions, see Crossley et al., 2000).

2.3.4. Tegu lizards

A 5 cm ventral incision was made through the sternum leaving the pericardium intact. For measurement of blood pressure a branch of the left pulmonary artery and right femoral artery was occlusively cannulated. A blood flow probe was placed around the common pulmonary vein.

2.3.5. Rattlesnakes

A small incision was made cranial to the heart and a PE50 catheter was advanced into the right aortic arch through the vertebral artery for measurement of P_{sys}. For measurement of P_{pul} a small branch of the pulmonary artery supplying the lower part of the lung was occlusively cannulated.. Blood flow probes were placed around the left aortic arch and the pulmonary artery. (For additional descriptions, see Galli et al., 2005b.)

In all cases, the 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. The pressure transducers were positioned at the level of the heart of the animal and were calibrated daily against a static water column. Flow probes were connected to a Transonic dual-channel blood flow meter (T206). Acoustical gel was infused around the blood flow probes to enhance the signal. Signals from the pressure transducer and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at 100 Hz.

2.4. Calculation of blood flows and vascular conductances

Because the left pulmonary artery was occlusively cannulated in the pythons, the measurement of blood flows in the right pulmonary artery represents total pulmonary blood flow (Q_{pul}). Total systemic blood flow (Q_{sys}) in the python has been estimated as 2.5 times blood flow in the left aortic arch (Q_{LAo}) (Skovgaard et al., 2005). In the turtle Q_{sys} has been estimated as $2.85 \times Q_{LAo}$ (Wang and Hicks, 1996). Rattlesnakes only have one pulmonary artery and measurements of blood flow in the pulmonary artery, therefore, represent Q_{pul}. Total systemic blood flow in the rattlesnake has been estimated as $3.3 \times Q_{LAo}$ (Galli et al., 2005b). Measurements of blood flow in the common pulmonary vein in the tegu lizard represent Q_{pul}. Total pulmonary blood flow in turtles and varanid lizards was estimated as 2 times blood flow in the left pulmonary artery, under the assumption that pulmonary blood flow is distributed evenly between the left and right pulmonary arteries.

When baseline blood flow changes more than baseline blood pressure, which is the case in most in vivo situations, conductance provides a better index than resistance for comparing vascular tone (Lautt, 1989; O'Leary, 1991). Pulmonary and systemic conductance (G_{pul} and G_{sys}, respectively) were calculated from mean blood flow and mean blood pressure ($G_{pul}=Q_{pul}/P_{pul}$ and $G_{sys}=Q_{sys}/P_{sys}$) assuming that central venous blood pressures are negligible.

2.5. Experimental protocol

After instrumentation, all variables were recorded for a period of 45 min to allow animal recovery from surgical stress and to obtain basal values. The animals received a sham injection of 0.9% w/v saline. All animals were treated with the nitric oxide donor sodium nitroprusside (SNP; 0.008, 0.08 and 0.2 mM) (varanid lizard and tegu lizard, 2.5 and 25 μg kg^{-1}; python, 60 μg kg^{-1}; turtle, 25 μg kg^{-1}; rattlesnake, 2.5 μg kg^{-1}) and effects upon hemodynamic variables were recorded. Following this, NO production was blocked by administration of the inhibitor of nitric oxide synthesis and L-arginine analogue, L-nitroarginine methyl ester (L-NAME; 0.2 M) (turtles, 50 mg kg^{-1}; varanid lizards, pythons, rattlesnakes, 150 mg kg^{-1}). All drugs were administered through an arterial catheter in 1 mL kg^{-1} aliquots and hemodynamic variables were allowed to return to baseline values between each injection.

2.6. Data analysis and statistics

Data were analyzed using AcqKnowledge data analysis software (version 3.7.1; Biopac, Goleta, CA). Mean blood pressures and flows were taken over a 2 min period prior to, and at the maximum effect of SNP and 20–30 min after injection of L-NAME when variables had stabilized. All data, when possible, are presented as means ± S.E. Differences in values before and after injection of SNP or L-NAME were assessed using paired *t*-tests. A limit for significance of $P<0.05$ was applied.

3. Results

3.1. Rattlesnakes

Fig. 2 shows all hemodynamic variables in one rattlesnake as well as mean hemodynamic values before and after injection of SNP (2.5 μg kg^{-1}) and L-NAME (150 mg

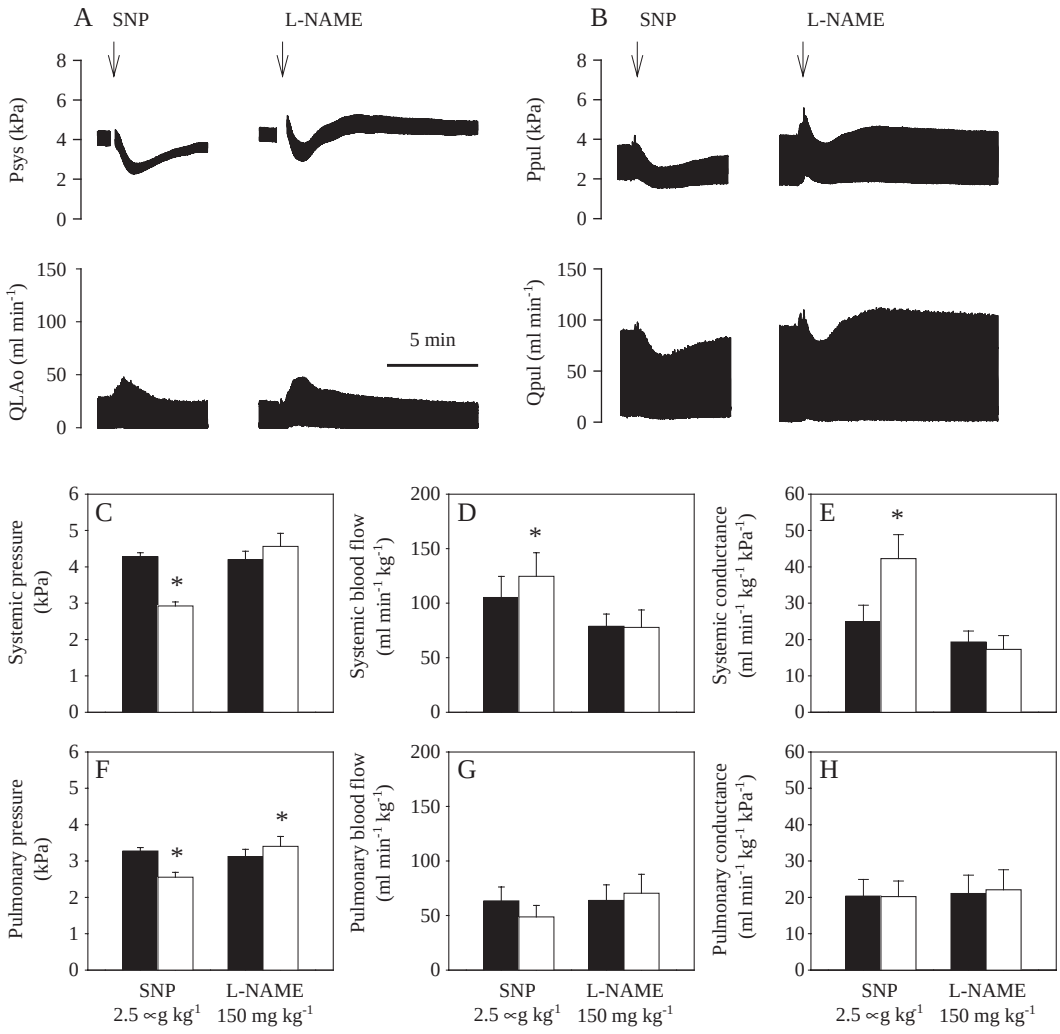

Fig. 2. Original traces of one rattlesnake, (A and B), and mean values of 6 individual experiments, (C–H), showing changes in the recorded variables following injection of SNP, sodium nitroprusside; 2.5 μg kg^{-1} and L-NAME, $N^{(1)}$nitro-L-arginine methyl ester; 150 mg kg^{-1}. Arrows indicate time of injection. P_{sys}, systemic arterial pressure; P_{pul}, pulmonary arterial pressure; Q_{LAo}, left aortic blood flow; Q_{pul}, pulmonary blood flow. Filled bars indicate pre-injection values and open bars indicate values following injection. Values are mean±S.E.M. * denotes a significant difference from pre-injection value ($P<0.05$).

kg^{-1}). SNP elicited a systemic vasodilatation, which is evident from the increased G_{sys} that was manifested as a decrease in P_{sys} and a rise in Q_{sys} (Fig. 2A and C–E). The systemic vasodilatation caused a pulmonary bypass of blood flow (right-to-left shunt), decreasing P_{pul} but G_{pul} was not affected (Fig. 2F–H). The immediate effects of injection of L-NAME were similar to those of SNP (Fig. 2A and B), but L-NAME only had very small effects on blood pressure when hemodynamic variables had stabilized (Fig. 2C–H).

3.2. Varanid lizards

The hemodynamic changes after injection of SNP (2.5 and 25 μg kg^{-1}) and L-NAME (150 mg kg^{-1}) in varanid lizards are shown in Fig. 3. In the systemic circulation, SNP increased G_{sys}, which lead to a reduction in P_{sys} and a rise in Q_{sys} Fig. 3A and C–E). In the pulmonary circulation, SNP caused an increase in G_{pul} and Q_{pul}, while P_{pul} remained relatively constant (Fig. 3B and F–H). L-NAME increased basal tone in the systemic circulation seen as a decrease in G_{sys} and an increase in P_{sys} (Fig. 3A and C–E) but had no significant effect on the pulmonary circulation (Fig. 3B and F–H).

3.3. Pythons

Fig. 4 shows the effect of SNP (60 μg kg^{-1}) and L-NAME (150 mg kg^{-1}) on the systemic and pulmonary circulation in pythons. SNP led to a large increase in G_{sys} and a large reduction in P_{sys}, while Q_{sys} was unaffected (Fig. 4A and C–E). G_{pul} increased in response to SNP, which elevated Q_{pul} but had no effect on P_{pul} (Fig. 4B and F–H). L-NAME decreased G_{sys} and increased P_{sys} while Q_{sys} remained constant (Fig. 4A and C–E). However, injection of L-NAME had no effect in the pulmonary circulation of the python (Fig. 4B and F–H).

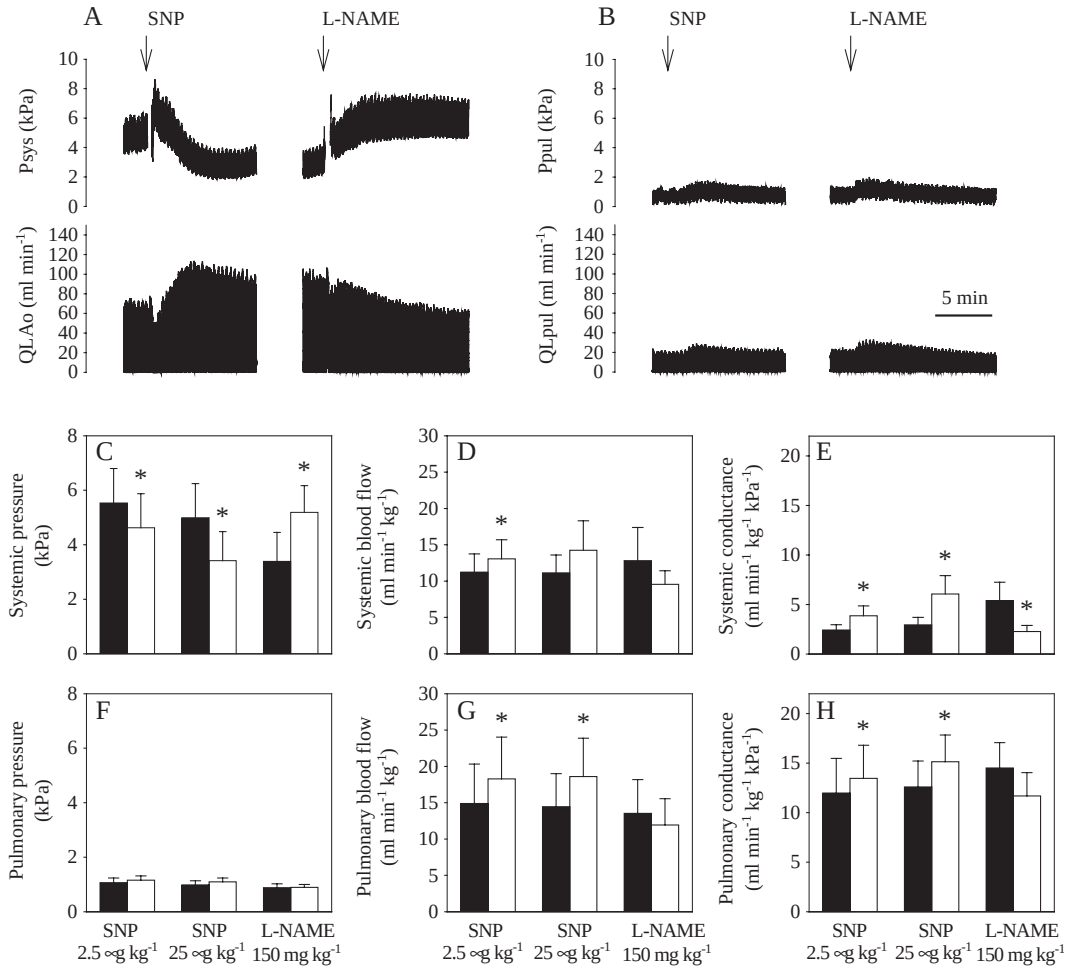

Fig. 3. Original traces of one varanid lizard, (A and B), and mean values of 6 individual experiments, (C–H), showing changes in the recorded variables following injection of SNP, sodium nitroprusside; 2.5 and 25 μg kg^{-1} and L-NAME, $N^{(1)}$nitro-L-arginine methyl ester, 150 mg kg^{-1}. Arrows indicate time of injection. P_{sys}, systemic arterial pressure; P_{pul}, pulmonary arterial pressure; Q_{LAo}, left aortic blood flow; Q_{Lpul}, left pulmonary blood flow. Filled bars indicate pre-injection values and open bars indicate values following injection. Values are mean ± S.E.M. * denotes a significant difference from pre-injection value ($P < 0.05$).

3.4. Turtles

Fig. 5 shows the effect of SNP (25 μg kg^{-1}) and L-NAME (50 mg kg^{-1}) on the systemic and pulmonary circulation in turtles (recalculated from original data, Crossley et al., 2000). Injection of SNP induced a systemic vasodilatation accompanied by a decrease in P_{sys} while Q_{sys} remained unchanged (Fig. 5A–C). SNP induced a large right-to-left-shunt decreasing P_{pul}, Q_{pul} causing a decrease in G_{pul} (Fig. 5D–F). Injection of L-NAME led to a systemic vasoconstriction with an increase in P_{sys} and decrease in Q_{sys} although the reduction in G_{sys} was not significant. G_{pul} was not affected, but P_{pul} increased (inducing a left-to-right-shunt) (Fig. 5).

3.5. Tegu lizards

Fig. 6 shows the changes in hemodynamic variables before and after injection of SNP (2.5 and 25 μg kg^{-1}) in one tegu lizard. Both dosages of SNP decreased P_{sys}, P_{pul} and Q_{pul} suggesting a systemic vasodilatation occurred. Similar responses were observed in an additional animal.

4. Discussion

4.1. The role of NO in the systemic circulation of reptiles

Injection of the exogenous nitric oxide donor SNP elicited a systemic vasodilatation, i.e. a rise in G_{sys}, in all of the five species of reptiles that have been studied so far. This clearly establishes a potential role for NO in regulating systemic vascular tone in reptiles. Administration of L-NAME to block constitutive NO synthesis, decreased G_{sys} in varanid lizards, pythons and turtles, while it had no effect in rattlesnakes. Continuous release of NO in the systemic circulation maintains basal systemic vascular tone in most species, but it seems that this contribution is minimal in rattlesnakes.

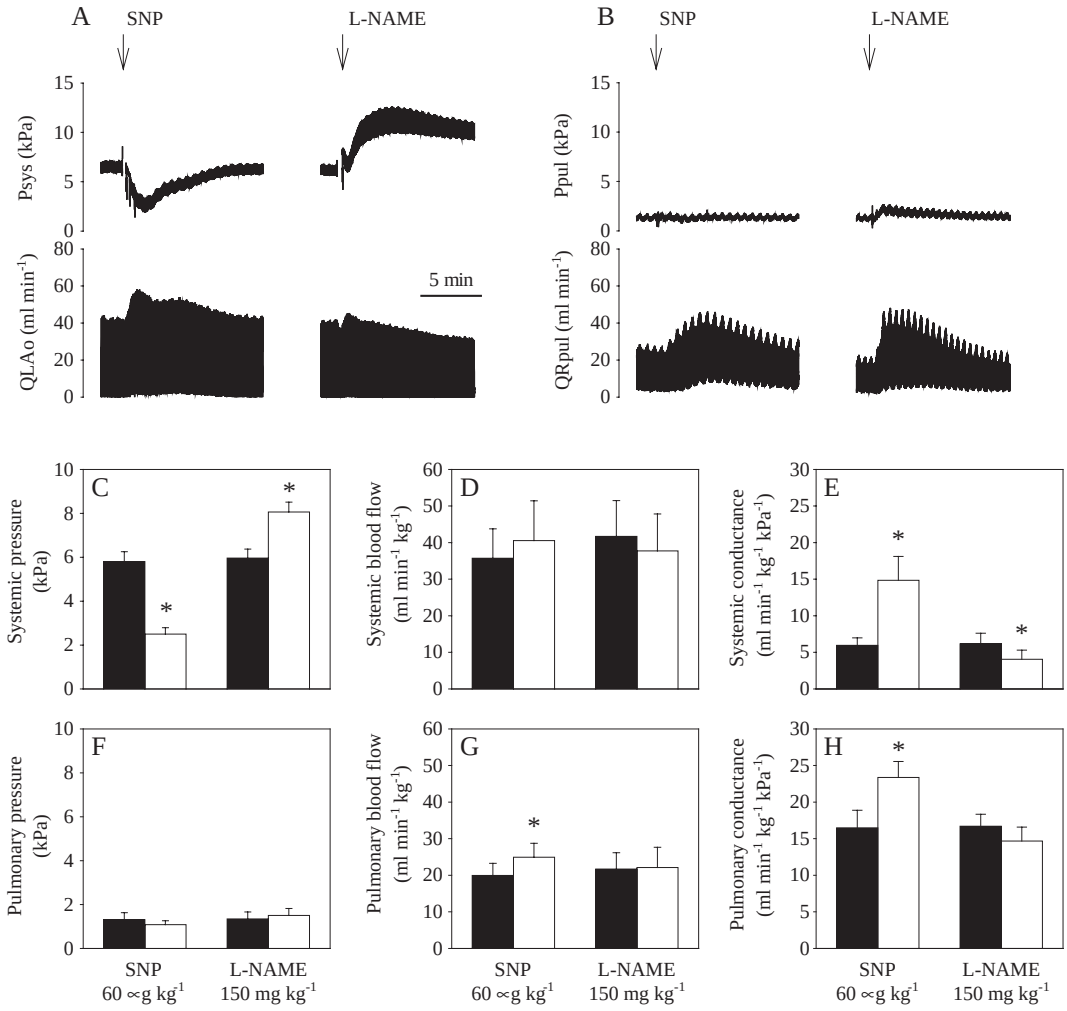

Fig. 4. Original traces of one python, (A and B), and mean values of 6 individual experiments, (C–H), showing changes in the recorded variables following injection of SNP, sodium nitroprusside; 60 μg kg^{-1} and L-NAME, $N^{(1)}$nitro-L-arginine methyl ester, 150 mg kg^{-1}. Arrows indicate time of injection. P_{sys}, systemic arterial pressure; P_{pul}, pulmonary arterial pressure; Q_{LAo}, left aortic blood flow; Q_{Rpul}, left pulmonary blood flow. Filled bars indicate pre-injection values and open bars indicate values following injection. Values are mean ± S.E.M. * denotes a significant difference from pre-injection value ($P<0.05$).

The finding that NO administration and inhibition of NOS affects overall hemodynamic variables in anaesthetized reptiles is consistent with studies on isolated vessels from other species of reptiles. In vitro, preconstricted strips of the aorta from the garter snake (*Thamnophis sirtalis parietalis*) relaxed in a dose-dependent manner in response to acetylcholine (ACh) and SNP (Knight and Burnstock, 1993). In the estuarine crocodile (*Crocodylus porosus*), the constriction of the aortic anastomosis in response to adrenaline, is counteracted by a reflex vasodilatation that is reduced after subsequent treatment with L-NAME (Axelsson et al., 2001).

Infusion of ACh into turtles following NOS blockade failed to increase cerebral blood flow (CBF) (Hylland et al., 1996). However, the elevation in CBF during hypercapnia or anoxia in the turtle (*T. scripta*) and during hypoxia in the estuarine crocodile (*C. porosus*) could not be abolished with NOS blockade (Hylland et al., 1996; Söderström et al., 1997, 1999). Thus, it seems that the ACh-induced cerebral vasodilatation may be due to NO, while the hypercapnic, anoxic and hypoxic induced increase in CBF is NOS independent (reviewed by Nilsson and Söderström, 1997).

In contrast to fish and amphibians, where recent anatomical investigations have shown that only nNOS is present, reptiles and endothermic vertebrates have both nNOS and eNOS (reviewed by Donald and Broughton, this issue). The responses to SNP and L-NAME revealed the presence of NO receptors in the systemic vasculature of the investigated species of reptiles. However, the use of SNP gives no information about the in vivo source of NO in the cardiovascular system of these reptiles, which could be either endothelium-derived or synthesized in neurons containing nNOS.

4.2. The role of NO in the pulmonary circulation of reptiles

In contrast to the clear role of NO in the systemic circulation the pulmonary vasculature seems far less

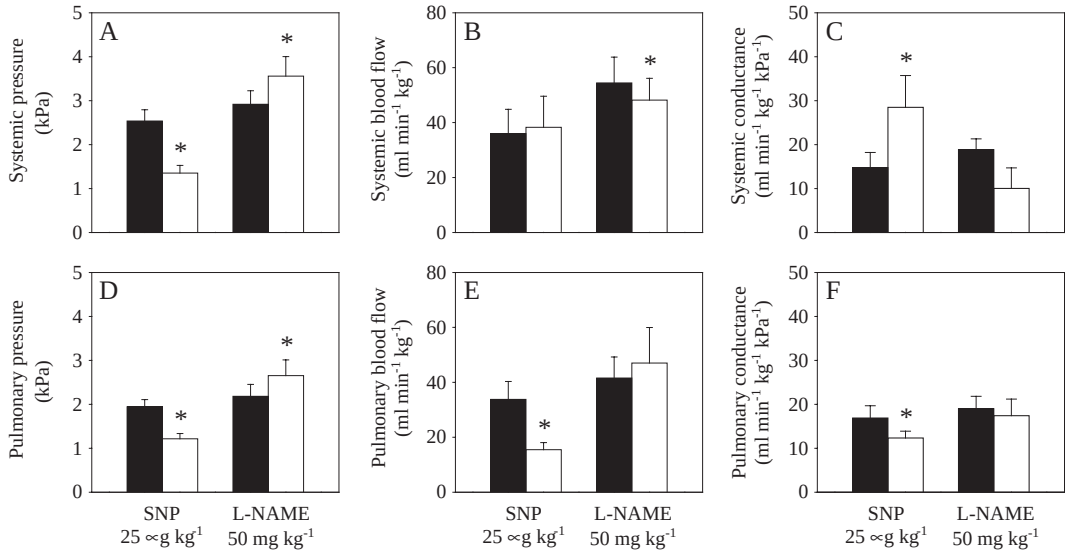

Fig. 5. Effects of injections of SNP, sodium nitroprusside; 25 μg kg^{-1} and L-NAME, $N^{(1)}$nitro-L-arginine methyl ester; 50 mg kg^{-1} in the turtle. Filled bars indicate pre-injection values and open bars indicate values following injection. Values are mean ± S.E.M. $N=5$. * denotes a significant difference from pre-injection value ($P<0.05$).

responsive. Thus, SNP did not affect G_{pul} in rattlesnakes or turtles and while there *was* a clear effect of SNP on G_{pul} in pythons and varanid lizards, indicating a potential role for NO in regulating pulmonary vasculature, these effects were smaller than the effects on the systemic vasculature. Treatment with L-NAME blocking nitric oxide synthesis

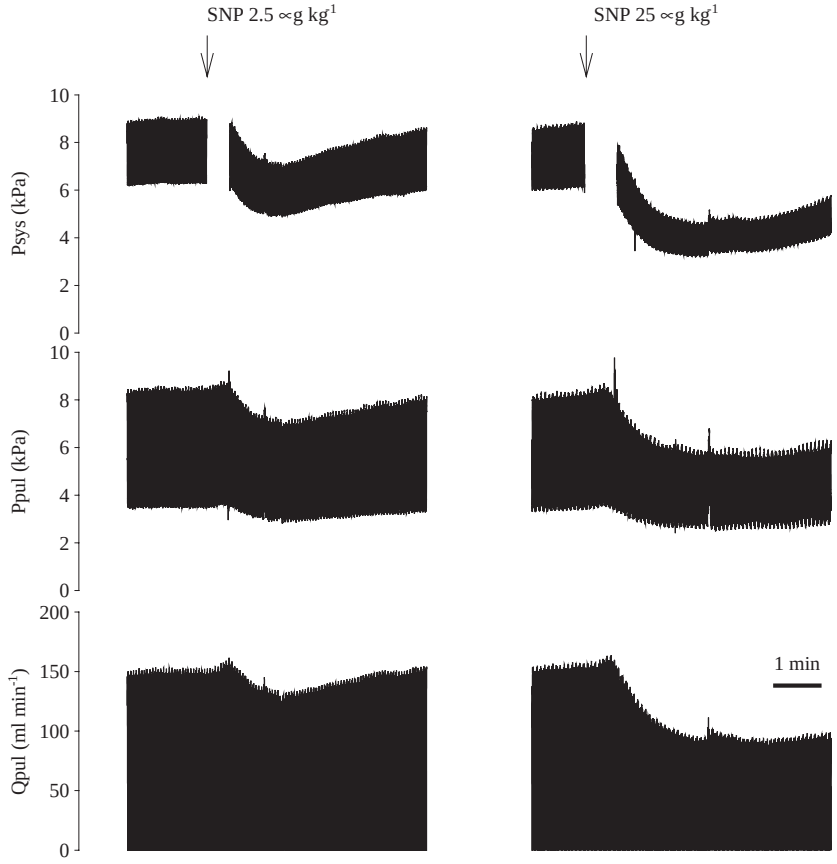

Fig. 6. Original traces of a tegu lizard showing changes in the recorded variables following injection of SNP, sodium nitroprusside, 2.5 μg kg^{-1} and 25 μg kg^{-1}. Arrows indicate time of injection. P_{sys}, systemic arterial pressure; P_{pul}, pulmonary arterial pressure and Q_{pul}, pulmonary blood flow.

had no effect in any species studied here, including pythons and varanid lizards, demonstrating a lack of contribution from NO in the maintenance of basal pulmonary vascular tone.

The extent to which NO contributes to the basal vascular tone in the low-pressure and low-resistance pulmonary circulation of mammals remains controversial (reviewed in Hampl and Herget, 2000). Some studies have reported a significant vasoconstriction following NOS inhibition (e.g. Cremona et al., 1994; DeWitt et al., 1997); whereas other studies report no changes (e.g. Barer et al., 1993; Cremona et al., 1994). Basal NO release increases, however, when pulmonary tone is elevated, which provides a protective mechanism avoiding pulmonary hypertension (Barnes and Liu, 1995). Thus, it has been suggested that NO plays an important role when the pulmonary vasculature is constricted during conditions such as hypoxic pulmonary vasoconstriction or pulmonary hypertension (Barnes and Liu, 1995). Clearly, it would be of interest to study the possible involvement of NO in the pulmonary circulation of reptiles during hypoxia.

In mammals, various humoral substances like ACh (McMahon et al., 1991, 1992), bradykinin (BK) (Ignarro et al., 1987), and substance P (SP) (Maggi et al., 1990; McMahon and Kadowitz, 1993) induce pulmonary vasodilatation through an endothelium-derived release of NO. Our study indicates a possible role for NO in regulating pulmonary conductance in the pythons and the varanid lizards. This is in contrast to earlier findings showing that the pulmonary vasculature in the python is virtually unresponsive to local regulating factors such as neuropeptide gamma (Skovgaard et al., 2005), neurotensin and endothelin-1 (N. Skovgaard, H. Nguyen, J.M. Conlon and T. Wang, unpublished observations).

4.3. Is nitric oxide more affective in species with higher vascular complexity or divided hearts?

All existing studies on reptiles clearly establish a potential role for NO in regulating vascular tone in the systemic circulation. Furthermore, NO may be important for maintaining basal systemic vascular tone in varanid lizards, pythons and turtles through a continuous release of NO. In contrast, the pulmonary circulation is less responsive to NO donors or NOS inhibitors, and it was only in pythons and varanid lizards that NO contributed to regulation of pulmonary vascular tone. Both species have a functionally divided circulation allowing for high mammalian-like systemic pressure and low pulmonary pressure, and it is possible that NO exerts a larger role in species with low pulmonary blood pressures, irrespective of the structural complexity of the lung (Fig. 1).

Important areas for future research are the role for NO in cardiovascular regulation in fully recovered reptiles at rest and during various physiological states, e.g. exercise, digestion, diving and thermoregulation. Furthermore, there is a need for further investigations of the role of NO in mediating responses to various regulatory peptides and its possible interactions with endothelin (Alonso and Radomski, 2003).

Acknowledgements

This study was supported by the Danish Research Council, the NOVO foundation and FAPESP. Gina Galli is in receipt of a special studentship from the BBSRC.

References

Alberola, A.M., Salazar, F.J., Nakamura, T., Granger, J.P., 1994. Interaction between angiotensin II and nitric oxide in control of renal hemodynamics in conscious dogs. Am. J. Physiol. 267, R1472–R1478.

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

Axelsson, M., Olsson, C., Gibbins, I., Holmgren, S., Franklin, C.E., 2001. Nitric oxide, a potent vasodilator of the aortic anastomosis in the estuarine crocodile, *Crocodylus porosus*. Gen. Comp. Endocrinol. 122, 198–204.

Barer, G., Emery, C., Stewart, A., Bee, D., Howard, P., 1993. Endothelial control of the pulmonary circulation in normal and chronically hypoxic rats. J. Physiol. (Lond.) 463, 1–16.

Barnes, P.J., Liu, S.F., 1995. Regulation of pulmonary vascular tone. Pharmacol. Rev. 47, 87–131.

Bennett, A.F., Ruben, J.A., 1979. Endothermy and activity in vertebrates. Science 206, 649–654.

Burggren, W., Johansen, K., 1982. Ventricular hemodynamics in the monitor lizard *Varanus exanthematicus*—pulmonary and systemic pressure separation. J. Exp. Biol. 96, 343–353.

Burggren, W., Farrell, A., Lillywhite, H., 1997. Vertebrate cardiovascular systems. In: Dantzler, W.H. (Ed.), Handbook of Physiology, Sec. 13, Comparative Physiology, vol. 1. American Physiological Society, Oxford, pp. 215–308.

Burnstock, G., 1990. Local mechanism of blood-flow control by perivascular nerves and endothelium. J. Hypertens. 8 (Suppl. 7), S95–S107.

Cai, H., Harrison, D.G., 2000. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ. Res. 87, 840–844.

Comeau, S., Lance, V.A., Hicks, J.W., Conlon, J.M., 1992. Purification and biological activity of alligator bradykinin. Am. J. Physiol. 263, R400–R404.

Conlon, J.M., Smith, D.D., Hicks, J.W., 1990. Isolation and biological activity of a novel kinin ([Thr^6]bradykinin) from the turtle, *Pseudemys scripta*. Endocrinology 126, 985–991.

Cremona, G., Wood, A.M., Hall, L.W., Bower, E.A., Higenbottam, T., 1994. Effect of inhibitors of nitric oxide release and action on vascular tone in isolated lungs of pig, sheep, dog and man. J. Physiol. (Lond.) 481, 185–195.

Crossley, D.A., Wang, T., Altimiras, J., 1998. Hypoxia elicits pulmonary vasoconstriction in anaesthetized turtles. J. Exp. Biol. 201, 3367–3375.

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

Delp, M.D., O'Leary, D.S., 2004. Integrative control of the skeletal muscle microcirculation in the maintenance of arterial pressure during exercise. J. Appl. Physiol. 97, 1112–1118.

DeWitt, B.J., Champion, H.C., Marrone, J.R., McNamara, D.B., Giles, T.D., Greenberg, S.S., Kadowitz, P.J., 1997. Differential effects of L-N^5-(1-iminoethyl)-ornithine on tone and endothelium-dependent vasodilator responses. Am. J. Physiol. 273, L588–L594.

Donald, J.A., Broughton, R.S., 2005. Nitric oxide of lower vertebrate blood vessels by vasomotor nerves. Comp. Biochem. Physiol. A. 142, 188–197 (this issue).

Förstermann, U., Closs, E.I., Pollock, J.S., Nakane, M., Schwarz, P., Gath, I., Kleinert, H., 1994. Nitric oxide synthase isozymes—characterization, purification, molecular cloning, and functions. Hypertension 23, 1121–1131.

Furchgott, R.F., Zawadski, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373–376.

Galli, G.L.J., Skovgaard, N., Abe, A.S., Taylor, E.W., Conlon, J.M., Wang, T., 2005a. Cardiovascular actions of rattlesnake bradykinin ([Val1Thr6]-bradykinin) in the anesthetized South American rattlesnake, *Crotalus durissus terrificus*. Am. J. Physiol. 288, R456–R465.

Galli, G., Skovgaard, N., Abe, A.S., Taylor, E.W., 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. 175B, 201–208.

Hampl, J., Herget, J., 2000. Role of nitric oxide in the parthogenesis of chronic pulmonary hypertension. Physiol. Rev. 80, 1337–1372.

Hicks, J.W., 1998. Cardiac shunting in reptiles: mechanism, regulation, and physiological functions. In: Gans, C., Gaunt, A.S. (Eds.), Biology of Reptilia, Morphology, vol. 19. Society for the study of amphibians and reptiles, Ithaca, NY, pp. 425–483.

Hylland, P., Nilsson, G.E., Lutz, P.L., 1996. Role of nitric oxide in the elevation of cerebral blood flow induced by acetylcholine and anoxia in the turtle. J. Cereb. Blood Flow Metab. 16, 290–295.

Ignarro, L.J., Byrnes, R.E., Buga, G.M., Wood, K.S., 1987. Mechanisms of endothelium-dependent vascular smooth relaxation elicited by bradykinin and VIP. Am. J. Physiol. 253, H1074–H1082.

Knight, G.E., Burnstock, G., 1993. Acethylcholine induces relaxation via the release of nitric oxide from endothelial cells of the garter snake (*Thamnophis sirtalis parietalis*) aorta. Comp. Biochem. Physiol. C. 106, 383–388.

Lautt, W.W., 1989. Resistance or conductance for expression of arterial vascular tone. Microvasc. Res. 37, 230–236.

Maggi, C.A., Pattachini, R., Perretti, F., Tramontana, M., Manzini, S., Geppetti, P., Santicioli, P., 1990. Sensory nerves, vascular endothelium and neurogenic relaxation of the guinea-pig isolated pulmonary artery. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 78–84.

McMahon, T.J., Kadowitz, P.J., 1993. Analysis of responses to substance P in the pulmonary vascular bed of the cat. Am. J. Physiol. 264, H394–H402.

McMahon, T.J., Hood, J.S., Bellan, J.A., Kadowitz, P.J., 1991. *N* omega-nitro-L-arginine methyl ester selectively inhibits pulmonary vasodilator responses to acetylcholine and bradykinin. J. Appl. Physiol. 71, 2026–2031.

McMahon, T.J., Hood, J.S., Kadowitz, P.J., 1992. Pulmonary vasodilator responses to vagal stimulation is blocked by *N* omega-nitro-L-arginine methyl ester in the cat. Circ. Res. 70, 364–369.

Miller, V.M., Vanhoutte, P.M., 1986. Endothelium-dependent responses in isolated blood vessels of lower vertebrates. Blood Vessels 23, 225–235.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Nilsson, G.E., Söderström, V., 1997. Comparative aspects on nitric oxide in the brain and its role as a cerebral vasodilator. Comp. Biochem. Physiol., A 118, 949–958.

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

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

Palmer, R.M., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524–526.

Perry, S.F., 1989. Mainstreams in the evolution of vertebrate respiratory structures. In: King, A.S., McMelland, J. (Eds.), Form and Function in Birds, vol. 4. Academic Press, London, pp. 1–67.

Piiper, J., 1992. Diffusion–perfusion inhomogeneity and alveolar–arterial O_2 diffusion limitation—theory. Respir. Physiol. 87, 349–356.

Piiper, J., 2000. Perfusion, diffusion and their heterogeneities limiting blood–tissue O_2 transfer in muscle. Acta Physiol. Scand. 168, 603–607.

Piiper, J., Scheid, P., 1991. Diffusion limitation of O_2 supply to tissue in homogenous and heterogenous models. Respir. Physiol. 85, 127–136.

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

Powell, F.L., Hopkins, S.R., 2004. Comparative physiology of lung complexity: implications for gas exchange. Newsl. Physiol. Sci. 19, 55–60.

Skovgaard, N., Galli, G., Taylor, E.W., Conlon, J.M., Wang, T., 2005. Hemodynamic effects of python neuropeptide γ in the anesthetized python, *Python regius*. Regul. Pept. 18, 15–26.

Söderström, V., Nilsson, G.E., Lutz, P.L., 1997. Effects of inhibition of nitric oxide synthesis and of hypercapnia on blood pressure and brain blood flow in the turtle. J. Exp. Biol. 200, 815–820.

Söderström, V., Nilsson, G.E., Renshaw, G.M.C., 1999. Hypoxia stimulates cerebral blood flow in the estuarine crocodile (*Crocodylus porosus*). Neurosci. Lett. 267, 1–4.

Wang, T., Hicks, J.W., 1996. Cardiorespiratory synchrony in turtles. J. Exp. Biol. 199, 1791–1800.

Wang, T., Smits, A.W., Burggren, W.W., 1998. Pulmonary function in reptiles. In: Gans, C., Gaunt, A.S. (Eds.), Biology of Reptilia, Morphology, vol. 19. Society for the study of amphibians and reptiles, Ithaca, NY, pp. 297–374.

Wang, T., Altimiras, J., Klein, W., Axelsson, M., 2003. Ventricular haemodynamics in *Python molurus*: separation of pulmonary and systemic pressures. J. Exp. Biol. 206, 4241–4245.

White, F.N., Hicks, J.W., Ishimatsu, A., 1989. Relationship between respiratory state and intracardiac shunts in turtles. Am. J. Physiol. 256, R240–R247.