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Hypoxia-induced vasoconstriction in alligator (*Alligator mississippiensis*) intrapulmonary arteries: a role for endothelin-1?

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SUMMARY

Hypoxic pulmonary vasoconstriction (HPV) is an adaptive response that diverts pulmonary blood flow from poorly ventilated and hypoxic areas of the lung to better ventilated parts, matching blood perfusion to ventilation. HPV is an ancient and highly conserved response expressed in the respiratory organs of all vertebrates. However, the underlying mechanism and the role of the endothelium remain elusive. Isolated intrapulmonary arteries (internal diameter <346 μm) from the American alligator *Alligator mississippiensis* were mounted in microvascular myographs for isometric tension recording. Resting vessels and vessels contracted with either serotonin (5-HT) or endothelin-1 (ET-1) were exposed to sustained (45 min) hypoxia (P_{O_2}<5 mmHg). In ET-1-contracted vessels, hypoxia induced a monophasic, sustained and fully reversible constriction, which was independent of the endothelium. In relaxed or in 5-HT-contracted vessels, hypoxia did not cause constriction. The effects of ET-1, ET_A and ET_B as well as the general ET-receptor antagonist were studied. ET-1 caused a contraction of the pulmonary arteries through stimulation of ET_A-receptors. ET_A and ET_B immunoreactive staining revealed the location of both receptors in the smooth muscle layer and of ET_B receptors in the endothelium. In conclusion, because precontraction with serotonin did not facilitate HPV, the required precontraction in alligators seems specific to ET-1, which implies that ET-1 plays an important permissive role for the HPV response in alligators.

Key words: reptile, alligator, hypoxic pulmonary vasoconstriction, endothelin-1, ET_A-receptor, ET_B-receptor, serotonin, noradrenaline.

INTRODUCTION

Hypoxic pulmonary vasoconstriction (HPV) is an adaptive response that diverts pulmonary blood flow from poorly ventilated and hypoxic areas of the lung to better ventilated parts (Von Euler and Liljestrand, 1946). HPV is considered important for local matching of blood perfusion to ventilation and improves pulmonary gas exchange efficiency (Von Euler and Liljestrand, 1946; Dawson, 1984; Brimioulle et al., 1996). The primary site of constriction is the small muscular resistance arteries (Weir and Archer, 1995). HPV of the mammalian lung is a locally mediated response and the hypoxic constriction persists in isolated and perfused lungs without neurohumoral influences, arterial pulmonary rings and even in isolated pulmonary arterial smooth muscle cells (PASMC) (Fishman, 1976; Madden et al., 1992; Ward and Aaronson, 1999). While the mechanism underlying HPV remains elusive (Aaronson et al., 2006), there is general consensus that hypoxia alters the production of reactive oxygen species (ROS) inhibiting voltage-gated K^+-channels, and that the resulting depolarisation of PASMC causes contraction as intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) rises (Moudgil et al., 2005; Aaronson et al., 2006). Numerous controversies, however, surround the role of the endothelium and the involvement of endothelin-1 (ET-1) (Aaronson et al., 2002). Thus, while several studies show that HPV is intrinsic to PASMC, other studies indicate that the endothelium, possibly through the release of ET-1, is essential for HPV (Madden et al., 1992; Shimoda et al., 2002; Robertson et al., 2003).

Hypoxic vasoconstriction is an ancient and highly conserved response expressed in the respiratory organs of all vertebrates, including lungs of mammals, birds and reptiles, amphibian skin and fish gills (Von Euler and Liljestrand, 1946; Faraci et al., 1984; Malvin and Walker, 2001; Olson et al., 2001; Skovgaard et al., 2005a). In mammals, ET-1 is recognised as a very potent and long-lasting vasoconstrictor of both the pulmonary and systemic circulation (Yanagisawa et al., 1988; Yanagisawa and Masaki, 1989; Cassin et al., 1991; Davenport et al., 1995) and evidence suggests a role for ET-1 in HPV and the pathophysiology of hypertension (Mateo and de Artiñano, 1997; Shimoda et al., 2002). ET-1 also exerts cardiovascular responses in ectothermic vertebrates (e.g. Olson et al., 1991; Poder et al., 1991; Wang et al., 1999; Wang et al., 2000; Hoagland et al., 2000; Platzack et al., 2002; Skovgaard et al., 2005b). In turtles and alligators, injections of ET-1 cause an initial and very pronounced dilation of the systemic vasculature which, in alligators, is followed by constriction. However, in the pulmonary circulation, ET-1 only has effects in the alligator where it constricts the vasculature (Platzack et al., 2002; Skovgaard et al., 2005b).

Hypoxia causes constriction of vascular smooth muscles in the systemic tissues of cyclostomes, which is independent of pretone and endothelium (Olson et al., 2001). This indicates that HPV is an ancient response intrinsic to the vasculature. However, as suggested by Olson et al. (Olson et al., 2001), it is possible that HPV has been embellished with secondary regulatory factors as vertebrates evolved to be more responsive to hypoxia, such that HPV in mammals has changed to a multifactorial process associated with several signalling pathways. Although controversial, reptiles appear to represent the earliest group of vertebrates where nitric oxide is released from the

endothelium (Donald and Broughton, 2005; Broughton and Donald, 2007) and it is likely that HPV in reptiles is mediated by intermediate mechanisms. Crocodilians exhibit a potent hypoxic pulmonary vasoconstriction *in vivo* (Skovgaard et al., 2005a) and the present study was designed to investigate the hypoxic responses of isolated intrapulmonary arteries of the American alligator (*Alligator mississippiensis*) as well as the putative role for ET-1 in hypoxia induced vasoconstriction.

MATERIALS AND METHODS

Experimental animals

Experiments were undertaken on 13 juvenile American alligators (*Alligator mississippiensis* Daudin 1803) of undetermined sex weighing between 0.29 and 1.55 kg (0.89±0.09 kg; mean ± s.e.m.). The animals were imported from Rockefeller Wildlife Refuge and shipped to the University of Aarhus, where they were kept in an aquarium containing water at 27°C and with access to dry platforms and basking lamps allowing for behavioural thermoregulation. All animals appeared healthy and grew considerably while kept in captivity. Food was withheld for 3 days prior to experiments. Experiments were performed according to Danish Federal Regulations.

Tissue preparation and mounting

Alligators were anaesthetised with isoflurane (Baxter, Allerød, Denmark), decapitated and pithed, so the lungs could be removed *en bloc* and placed in cold physiological salt solution (PSS): (mmol l^{-1}) 119 NaCl, 25 $NaHCO_3$, 1.18 KH_2PO_4, 4.7 KCl, 1.17 $MgSO_4$, 1.6 $CaCl_2$ and 5.5 glucose. Intrapulmonary muscular resistance arteries (78<i.d.<346 μm) were dissected from the anterior part of the right lung and mounted on a wire myograph (Model 410A, Danish Myo Technology, Aarhus, Denmark) for recording of isometric tension (Mulvany and Halpern, 1977) using a PowerLab data acquisition system (ADInstruments, Oxfordshire, UK). The vessels were immersed in 10 ml PSS heated to 25°C and aerated with 3% CO_2 and 97% room air (pH ~7.3) delivered by a gas mixing pump (Wösthoff, Bochum, Germany). Then vessels were left for 30 min to stabilise and resting tension was normalised by adjusting the diameter of the vessel with a micrometer screw to a transmural pressure of 1.5 kPa, as measured in American alligators (Jones and Shelton, 1993). The vessels were then left for additional 30 min before the experimental protocol commenced.

Experimental protocol

Contractility of all vessels was evaluated by replacement of PSS with a high K^+ solution (KPSS 60 mmol l^{-1}, which is PSS with NaCl substituted by KCl on an equimolar basis). The presence of an intact endothelium was assessed by addition of acetylcholine, to a final bath concentration of 10^{-5} mol l^{-1}, which relaxes the vessels through release of nitric oxide from the endothelium. After end protocol all vessels were fixed for Haematoxylin/Eosin staining to verify an intact endothelium.

In the search for a suitable preconstrictor of the alligator pulmonary arteries, concentration–response curves for several well-known constricting agents in mammalian vessels were obtained. The effect of cumulative addition of serotonin (5-HT, 10^{-10}–3×10^{-6} mol l^{-1}) and endothelin-1 (ET-1, 10^{-10}–10^{-7} mol l^{-1}) on relaxed vessels were studied. Furthermore, effects of noradrenaline (NA, 10^{-10}–10^{-5} mol l^{-1}) on relaxed vessels and on vessels preconstricted with ET-1 (10^{-8} mol l^{-1}) were investigated; this was done before and after incubation with propranolol (10^{-5} mol l^{-1}) for 20 min.

The effects of hypoxia were studied by changing the gas mixture supplied to the experimental chamber from normoxia (3% CO_2 in 97% air) to hypoxia [3% CO_2 and 97% N_2, P_{O_2}<5 mmHg (Radiometer, Copenhagen, Denmark)] for 45 min and returned to normoxia. This was performed on relaxed vessels (baseline), vessels preconstricted with 5-HT (3×10^{-6}–3×10^{-8} mol l^{-1}) and vessels preconstricted with ET-1 (10^{-8}–3×10^{-8} mol l^{-1}) with an intact endothelium and after removal of the endothelium. The endothelial layer was removed by introducing a hair straw into the vessel and rubbing forth and back several times. Vessels were fixed for Haematoxylin/Eosin staining to verify successful removal of the endothelium.

The effects of ET-1 (10^{-10}–10^{-7} mol l^{-1}) in the presence of the specific ET_A-receptor antagonist BQ-123 (3×10^{-6} mol l^{-1}), the specific ET_B-receptor antagonist BQ-788 (3×10^{-6} mol l^{-1}) or the general ET-receptor antagonist tezosentan (10^{-5} mol l^{-1}) were studied. Upon completion of the protocol, all vessels were fixed for immunohistochemical studies.

All chemicals were purchased from Sigma-Aldrich (Brøndby, Denmark) except for tezosentan, which was a generous gift from Actelion Pharmaceuticals (Allschwil, Switzerland).

Immunohistochemistry

To study the presence and localization of ET_A- and ET_B-receptors, vessels were fixed in cold (4°C) 4% formaldehyde (pH 7.0) for 1 h, then stored in 50% alcohol until embedded in paraffin, after which longitudinal sections of 3 μm were obtained. After de-waxing and rehydration, antigen retrieval was achieved through heat exposure (microwave 600 W, 2×5 min) of sections immersed in TEG-buffer (Tris 10 mmol l^{-1}, EGTA 0.5 mmol l^{-1}, pH 9.0) followed by a wash in PBS (phosphate-buffered saline, pH 7.1, 2×5 min). To prevent unspecific binding of antibodies, segments were incubated with 10% fetal bovine serum for 20 min. Segments were then incubated 24 h (4°C) with either rabbit anti-endothelin A receptor antibody (1:500, Sigma-Aldrich, Brøndby, Denmark) or rabbit anti-endothelin B receptor antibody (1:250, Sigma-Aldrich, Brøndby Denmark), diluted with 1% bovine serum albumin (BSA) in PBS. Both antibodies were raised against synthetic peptide receptor fragment. Negative controls for non-specific staining were obtained by replacing primary antibodies with 1% BSA. Sections of rat lung tissue were included as positive controls. After the 24 h incubation period with primary antibodies and wash in PBS, sections were incubated in the dark for 1 h with the secondary flourescein isothiocyanate (FITC) conjugated antibody (1:400, goat anti-rabbit IgG, Alexa Flour® 488, Invitrogen, Taastrup, Denmark). Finally, sections were washed in PBS, dehydrated and mounted with anti-fade fluorescent medium (DakoCytomation, Glostrup, Denmark). Stained sections were examined under a confocal microscope (LSM 510 META, Zeiss, New York, NY, USA) with a 488 nm laser line and 505–550 nm emission filter.

Haematoxylin/Eosin staining

To verify intact endothelium or successful removal of endothelium, the fixated vessels were paraffin embedded and sectioned as described above. Sections were stained with Haematoxylin and Eosin and examined under a Zeiss light microscope (Zeiss, New York, NY, USA).

Data analysis and statistics

The mechanical response of the vessel segments was measured as active wall tension (ΔT), which is the change in force (ΔF)

divided by twice the segment length (2*l*) (Mulvany and Halpern, 1977). Contraction is expressed relative to the contraction induced by 60 mmol l^{-1} KPSS and relaxation is given as a percentage of the preconstriction. All data recordings were analyzed using Chart5^{TM} software (ADInstruments, Oxfordshire, UK). A one-way ANOVA for repeated measures followed by Dunnett's *post hoc* test or a two-way ANOVA followed by a Tukey *post hoc* test when appropriate were applied to evaluate significant differences. Differences were considered statistically significant at a 95% level of confidence (P<0.05). All data are presented as mean ± s.e.m.

RESULTS

5-HT and endothelin induced concentration-dependent vasoconstrictions of the alligator pulmonary arteries (Fig. 1A,B). In contrast, NA evoked a concentration-dependent relaxation in ET-1 preconstricted vessels, and NA relaxation was markedly reduced in the presence of the β-adrenoceptor antagonist propranolol (Fig. 1C).

In ET-1 contracted pulmonary arteries, hypoxia induced a superimposed monophasic, sustained and fully reversible contraction (Fig. 2). Hypoxia, however, did not affect tension in the absence of preactivation or in vessels contracted with 5-HT (Fig. 3). There were no differences in the levels of preconstriction with 5-HT and ET-1 (P=0.153, N=6). In ET-1-contracted vessels, hypoxia induced vasoconstriction both in segments with and without endothelium increasing vascular tone 171±82% and 71±25% (P=0.162, N=6), respectively (Fig. 3).

Incubation with the ET_B-receptor antagonist BQ-788 did not change the concentration–response curve for ET-1 (Fig. 4). However, incubation with the ET_A-receptor antagonist BQ-123 right shifted the curve and the general ET-receptor antagonist tezosentan abolished the effect of ET-1 (Fig. 4). In alligator intrapulmonary arteries, strong ET_A-immunoreactivity and weaker ET_B-immunoreactivity were observed in media and adventitia as well as endothelial cells (Fig. 5). In rat control tissue ET_A- and ET_B-immunoreactivity were also seen in media, adventitia and endothelial cells (data not shown).

DISCUSSION

Hypoxia caused a superimposed monophasic, sustained (45 min) and fully reversible constriction in ET-1-contracted alligator intrapulmonary arteries. This resembles the *in vivo* HPV response in caimans (*Caiman latirostris*), where hypoxia induces a reversible sustained monophasic contraction of the pulmonary vasculature (Skovgaard et al., 2005a). In the alligator, the hypoxic constriction depended on pretone of the vessels; in vessels preconstricted with ET-1 hypoxia induced a constriction, whereas relaxed vessels and vessels preconstricted with 5-HT did not respond to hypoxia. The dependence of pretone seems to differ within mammals and HPV can be elicited in the presence of several constrictive agonists in species where a low level of preconstriction is required (Aaronson et al., 2006). It has been argued that pretone is required to induce a sub-threshold depolarisation of the smooth muscle cells (SMC) to ensure that the hypoxia stimulated depolarisation is sufficient to activate calcium influx (Ward and Aaronson, 1999). This sub-threshold depolarisation may reinstate the *in vivo* basal vascular tone, which is normally maintained by circulating or endothelial-derived vasoactive substances. Because preconstriction with serotonin did not facilitate HPV, the required pretone of alligators seems specific to ET-1, and not merely an unspecific depolarisation. This also implies that ET-1 plays an important role for the HPV response in alligators.

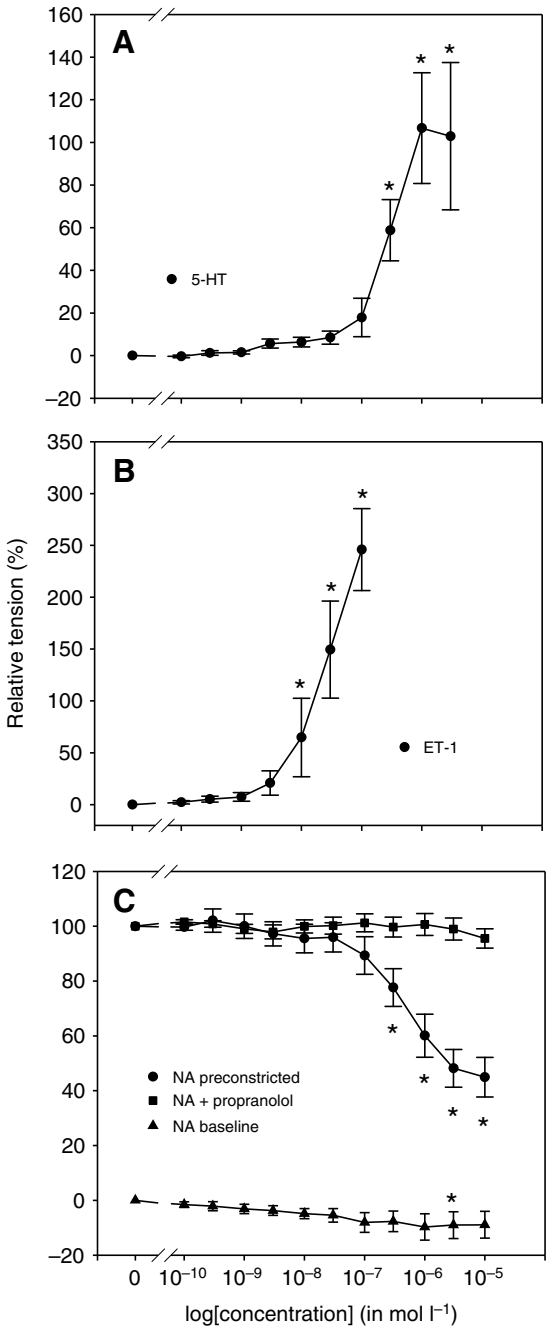

Fig. 1. Effects of increasing concentration of serotonin (5-HT; A), endothelin-1 (ET-1; B) and noradrenaline (NA; C), on the relative tension of alligator intrapulmonary arteries. The effects of NA were evaluated on relaxed vessels as well as vessels preconstricted with ET-1 (10^{-8} mol l^{-1}) before and after incubation with propranolol (10^{-5} mol l^{-1}). Contraction is expressed relative to the contraction induced by 60 mmol l^{-1} KPSS and relaxation is given as a percentage of the precontraction. Values are means ± s.e.m.; each dose–response relationship is based on six blood vessels from six different animals, using a total of 13 alligators. An asterisk indicates a significant difference (P<0.05) from control values evaluated by a one-way ANOVA for repeated measures or a two-way ANOVA followed by Dunnett's *post hoc* test.

ET-1 caused a dose-dependent constriction of the alligator pulmonary arteries. The constriction was strongly attenuated by blockade of ET_A-receptors and was abolished by the general ET-

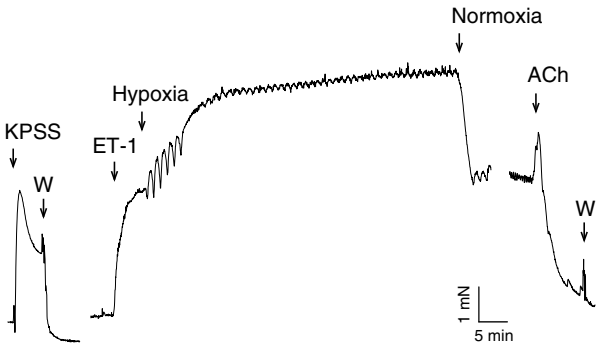

Fig. 2. Original tension recordings showing the effects of hypoxia (P_{O_2}<5 mmHg) on an alligator intrapulmonary artery, internal circumference 1086 μm, preconstricted with endothelin-1 (ET-1; 10^{-8} mol l^{-1}). The hypoxic constriction is compared to the constriction induced by KPSS (60 mmol l^{-1}). The degree of relaxation with acetylcholine (ACh; 10^{-5} mol l^{-1}) verifies an intact endothelium; W, wash with PSS.

antagonist showing that the constriction was mediated through ET_A-receptors. Within reptiles, the role of the ET-receptors has been addressed in only a few studies; in turtles the systemic dilation is mediated through ET_B-receptors (Skovgaard et al., 2005b), and in snakes stimulation of ET_A-receptors causes a constriction of the aorta (Borgheresi et al., 2006). The amino acid sequence of alligator ET-1 is identical to that of mammals (Platzack et al., 2002). Furthermore, the partial sequence of the ET_A-receptor from another reptile (*Bothrops jararaca*) shows a very high sequence similarity with ET_A-receptor sequences from chicken, rat, human and *Xenopus* (Borgheresi et al., 2006), which strengthened the case for use of heterologous antibodies in this study. ET_A- and ET_B-immunoreactivity indicated that both subtypes of receptors were present in the muscular layer as well as the endothelium in the alligator intrapulmonary arteries. Mammalian ET_B-receptors are found both within the smooth muscles where stimulation causes constriction, and in the endothelium where stimulation leads to dilation through the release of nitric oxide and prostacyclins (e.g. Mateo and de Artiñano, 1997; Masaki, 2004). Mammalian ET_A-receptors, however, are located only within the smooth muscles where stimulation causes constriction (e.g. Mateo and de Artiñano, 1997; Masaki, 2004), and the findings in the present study agree with these observations, since ET_A-immunoreactivity was strong in the smooth muscle layer of alligator intra-pulmonary arteries. Thus, mainly smooth muscle ET_A-receptors contribute to the ET-1 mediated contraction.

The role of the endothelium and ET-1 in HPV

When the pulmonary arteries were preconstricted with ET-1, the hypoxic constriction was independent of the endothelium in alligators. The role of the endothelium in HPV of mammals remains controversial. Thus, isolated pulmonary arteries without endothelium and freshly isolated PASMC from mammals, but not systemic arterial SMC, contract in response to hypoxia (Madden et al., 1992; Wang et al., 1995; Aaronson et al., 2002). This would indicate that HPV is intrinsic to the SMC, which, in that case, can sense oxygen, depolarise, increase $[Ca^{2+}]_i$ and contract in response to hypoxia independent of the endothelium. Nevertheless, other studies have shown that the hypoxic contraction is endothelium dependent (Aaronson et al., 2002; López-Valverde et al., 2005). Removal of the endothelium from rat pulmonary arteries does not suppress the rise in $[Ca^{2+}]_i$ during sustained hypoxia, but abolishes the hypoxic contraction, suggesting that the endothelium releases a factor that sensitises the contractile apparatus of the SMC to calcium enabling hypoxic vasoconstriction (Robertson et al., 2003).

The hypoxic vascular response of alligator intrapulmonary arteries may not be endothelium-independent as much as it is ET-1-dependent. It has long been thought that ET-1 is released from the endothelium during hypoxia, causing the actual constriction of the vascular smooth musculature (VSM). However, the response of the ET-1 constriction is notorious for its sustained and often irreversible constriction in pulmonary arteries, which does not resemble the hypoxic constriction and its fast reversal (Vanhoutte et al., 1989). When preconstricted with ET-1, HPV was superimposed on the stable contraction with ET-1 in the alligator vessels indicating that the two responses were mediated through different pathways. It has been suggested that ET-1, and hence

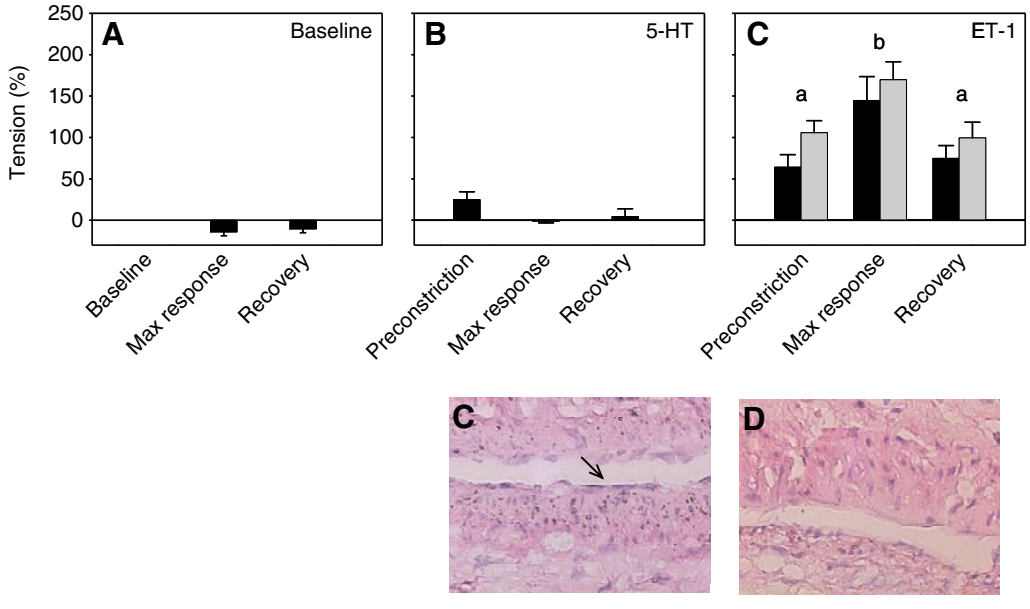

Fig. 3. Effects of hypoxia (45 min, $\dot{P}_{O_2}$<5 mmHg) on alligator intrapulmonary arteries. (A) Relaxed vessels (baseline), (B) vessels preconstricted with serotonin (5-HT) and (C) vessels preconstricted with endothelin-1 (ET-1) with an intact endothelium (black bars) or denuded (grey bars). Contraction is expressed relative to the contraction induced by 60 mmol l^{-1} KPSS. Values are means ± s.e.m., *N*=3 (A) and *N*=6 (B,C). Different letters indicate a significant difference (P<0.05) from baseline evaluated by a two-way ANOVA followed by a Tukey *post hoc* test. Haematoxylin/Eosin staining shows the presence (D) and absence (E) of endothelium in representative vessels (40× magnifications). The arrow shows endothelial cells.

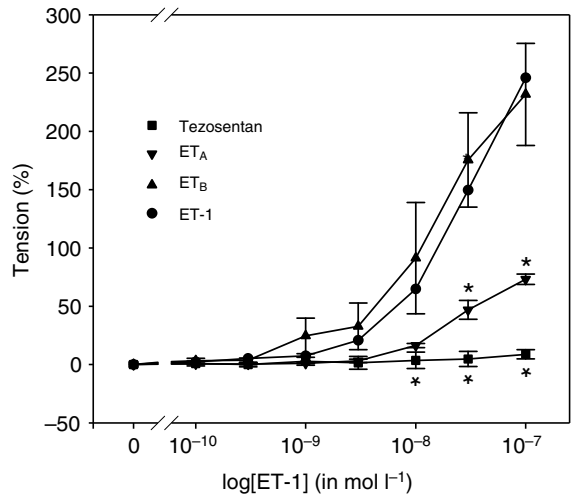

Fig. 4. Effects of increasing concentrations of endothelin-1 (ET-1) on the relative tension of alligator intrapulmonary arteries: non-treated vessels, vessels incubated with either the specific ET_A-receptor antagonist BQ-123 (3×10^{-6} mol l^{-1}), the specific ET_B-receptor antagonist BQ-788 (3×10^{-6} mol l^{-1}) or the general ET-receptor antagonist tezosentan (10^{-5} mol l^{-1}). Contraction is expressed relative to the contraction induced by 60 mmol l^{-1} KPSS. Values are means ± s.e.m., *N*=7 (ET-1) and *N*=4 (ET_B, ET_A and tezosentan). An asterisk denotes a significant difference (*P*<0.05) from control treatment with ET-1 alone, evaluated by a two-way ANOVA followed by a Tukey *post hoc* test.

the endothelium, may serve a permissive role in HPV enabling the response of the SMC (Shimoda et al., 2002). In isolated PASMC there was a substantial increase in the extent of hypoxic contraction after addition of ET-1 in a concentration that did not alter cell length or $[Ca^{2+}]_i$ (Sham et al., 2000). Also, in vessels without endothelium, where HPV was abolished, the hypoxic response was restored upon addition of ET-1 (Liu et al., 2001). Moreover, a study in chronically hypoxic rats showed that ET-1 serves the priming role of sensitising the contractile apparatus through stimulation of Rho-kinase (Weigand et al., 2006). The permissive role of ET-1 in HPV may also be through suppression of K_{ATP}-channels (Sato et al., 2000). Thus, HPV may be intrinsic for the SMC but ET-1 required for the full *in vivo* expression of the hypoxic vascular response.

Adrenergic and serotonergic regulation of pulmonary blood flow

Pulmonary blood flow of reptiles is largely regulated by the autonomic nervous system through an adrenergic dilation in crocodilians, and a cholinergic constriction in non-crocodilian reptiles, of the proximal pulmonary artery (e.g. Milsom et al., 1977; Franklin and Axelsson, 2000). Although there is a substantial adrenergic innervation of the intrapulmonary vasculature in reptiles (Donald and Lillywhite, 1989; Donald et al., 1990), the functional significance of the sympathetic nerves on pulmonary vasculature and control of blood flow remains uncertain (Overgaard et al., 2002; Galli et al., 2007). Our study clearly demonstrates that NA dilates intrapulmonary arteries in alligators through stimulation of β-adrenoceptors. Thus, although autonomic regulation of the proximal pulmonary artery is the primary determent of pulmonary blood flow, adrenergic innervations may play an important role in local or regional regulation of blood flow within the lung. 5-HT caused a concentration-dependent constriction of the alligator intrapulmonary arteries revealing a potential role in regulating pulmonary blood flow. This is in conjunction with the identification of 5-HT immunoreactive cells in the pulmonary vasculature in the lungs of file snakes (Donald and Lillywhite, 1989). The effects of 5-HT and ET-1 in the pulmonary circulation of alligators are in contrast to the very small or lacking effects of various regulatory peptides and nitric oxide in the pulmonary circulation of most reptiles (Skovgaard and Wang, 2006).

In conclusion, our study shows that, hypoxia constricts the intrapulmonary arteries of alligators. This HPV is monophasic, sustained and reversible and resembles that observed *in vivo*. The constriction appears to be dependent on the presence of ET-1. ET-1 constricts the intrapulmonary alligator arteries through stimulation of the ET_A-receptors, mainly in the smooth vasculature.

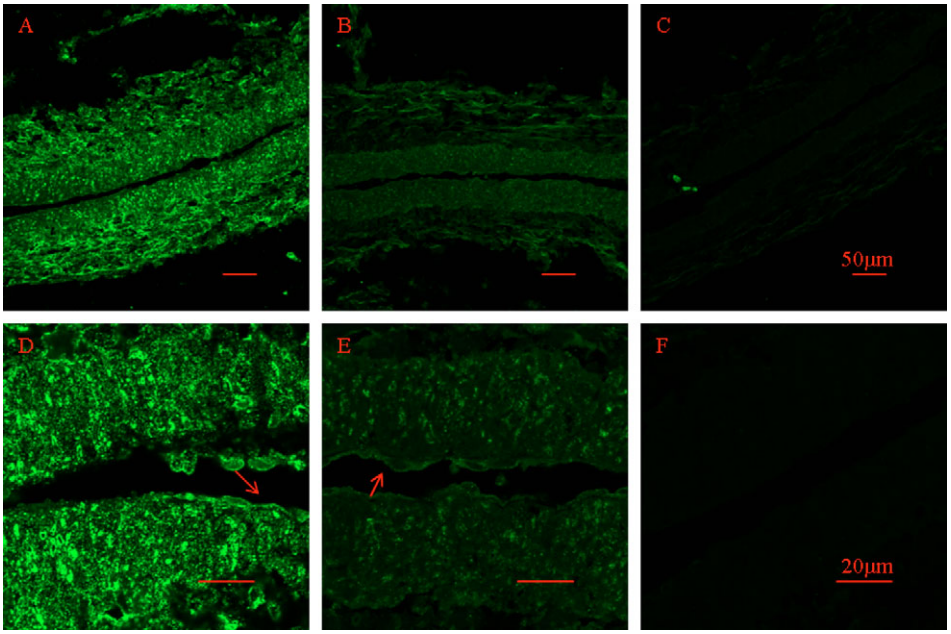

Fig. 5. Representative micrographs showing immunofluorescent staining with anti-ET_A (A,D) and anti-ET_B (B,E) receptor antibodies in longitudinal sections of an alligator intra-pulmonary artery (internal circumference: 586 μm). (C,F) Negative controls. (A,B) strong staining for ET_A-receptors and weaker staining for ET_B-receptors in media and adventitia (20× magnification). (D,E) strong staining for ET_A-receptors and weaker staining for ET_B-receptors in endothelial cells (40× magnification). Arrows in D,E indicate epithelial cells.

LIST OF ABBREVIATIONS

5-HT	serotonin
BSA	bovine serum albumin
ET-1	endothelin-1
FITC	flourescein isothiocyanate
HPV	hypoxic pulmonary vasoconstriction
KPSS	high potassium PSS
NA	noradrenaline
PASMC	pulmonary arterial smooth muscle cells
PBS	phosphate-buffered saline
PSS	physiological salt solution
ROS	reactive oxygen species
SMC	smooth muscle cell
VSM	vascular smooth musculature

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