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Hemodynamic effects of python neuropeptide γ in the anesthetized python, *Python regius*

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Abstract

The effects of python neuropeptide gamma (NPγ) on hemodynamic parameters have been investigated in the anesthetized ball python (*Python regius*). Bolus intra-arterial injections of synthetic python NPγ (1–300 pmol kg^{-1}) produced a dose-dependent decrease in systemic arterial blood pressure (P_{sys}) concomitant with increases in systemic vascular conductance (G_{sys}), total cardiac output and stroke volume, but only minor effects on heart rate. The peptide had no significant effect on pulmonary arterial blood pressure (P_{pul}) and caused only a small increase in pulmonary conductance (G_{pul}) at the highest dose. In the systemic circulation, the potency of the NK1 receptor-selective agonist [Sar^9,$Met(O_2)^{11}$)] substance P was >100-fold greater than the NK2 receptor-selective agonist [βAla^8] neurokinin A-(4–10)-peptide suggesting that the python cardiovascular system is associated with a receptor that resembles the mammalian NK1 receptor more closely than the NK2 receptor. Administration of the inhibitor of nitric oxide synthesis, L-nitro-arginine-methylester (L-NAME; 150 mg kg^{-1}), resulted in a significant ($P<0.05$) increase in P_{sys} as well as a decrease in G_{sys}, but no effect on P_{pul} and G_{pul}. Conversely, the nitric oxide donor, sodium nitroprusside (SNP; 60 μg kg^{-1}) produced a significant ($P<0.05$) decrease in P_{sys} along with an increase in G_{sys} and pulmonary blood flow. However, neither L-NAME nor indomethacin (10 mg kg^{-1}) reduced the cardiovascular responses to NPγ. Thus, nitric oxide is involved in regulation of basal vascular tone in the python, but neither nitric oxide nor prostaglandins mediate the vasodilatory action of NPγ.

Keywords: Squamate; Tachykinin; Nitric oxide; Cardiovascular

1. Introduction

Tachykinins are a family of structurally-related neuropeptides that are synthesized in neurons of the central and enteric nervous systems as well as the dorsal root ganglia. Tachykinins are involved in signal transduction in sensory nerves, in the regulation of gastrointestinal motility and pulmonary function, and exert a wide range of cardiovascular effects. It has been proposed that they are particularly important in controlling the increased blood flow to the gastrointestinal organs after feeding (postprandial hyperemia) [13,29].

In mammals, the tachykinins are represented by substance P (SP), neurokinin A (NKA), neuropeptide γ (NPγ) and neuropeptide K, that are encoded by the preprotachykinin A gene, by neurokinin B, encoded by the preprotachykinin B gene, and by hemokinin-1 encoded by the preprotachykinin-C gene (reviewed in Ref. [6]). The preprotachykinin A gene directs the synthesis of at least four biosynthetic precursors of the tachykinins (α-, β-γ- and δ-preprotachykinin A) that arise from the primary transcription product by an alternative RNA splicing mechanism. NPγ is a 21 amino-acid-residue peptide, containing the amino acid sequence of NKA at its COOH-terminus, that is a specific product of the post-translational processing of γ-preprotachykinin A [6,9].

NPγ was first isolated from an extract of the intestine of the rabbit [11] and subsequently from the brains of the

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American alligator [31] and desert tortoise [30], from the intestine of the Burmese python [7], and from the gastrointestinal tissues of a diverse range of fish species [goldfish, rainbow trout, bowfin, pallid sturgeon, and hammerhead shark] (reviewed in Ref. [30]). Although the amino acid sequence of NPγ in human, ox and rat is the same as that in the rabbit, the primary structure of NPγ has been poorly conserved during evolution of the non-mammalian vertebrates. Only those amino acid residues at the extreme COOH-terminal sequence of the peptide (Phe-Val-Gly-Leu-Met NH_2) are invariant in all species. Among the reptiles, turtle and alligator NPγ are identical, but python NPγ shows three substitutions (Gly^5→Ser, Gln^6→Pro and Ile^7→Leu) compared with alligator NPγ and an additional substitution (His^4→Tyr) compared with mammalian NPγ.

NPγ causes vasodilatation of both the systemic and pulmonary circulations of mammals [23,32,33], but the hemodynamic effects of NPγ have never been investigated in a non-mammalian species. We have previously shown that python SP is an extremely potent vasodilator in the systemic circulation of the python [27], but nothing is known about the effect of a tachykinin on the pulmonary circulation in any reptile. As with other reptiles that can ingest large meals, pythons are characterized by very large cardiovascular responses to the elevated metabolic rate during digestion [21,28,29]. Furthermore, pythons are exceptional amongst reptiles by possessing a functionally divided heart that allows for low blood pressures in the pulmonary circulation while maintaining mammalian-like pressures in the systemic circulation [25,26]. In the present study, we describe the effects of bolus intra-arterial injections of a synthetic replicate of python NPγ on the systemic and pulmonary circulations of the anesthetized ball python (*Python regius*).

2. Materials and methods

2.1. Peptide synthesis

Python NPγ (Asp-Ala-Gly-Tyr-Ser-Pro-Leu-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2) was synthesized by solid-phase methodology on a 0.025 mmol scale using an Applied Biosystems (Foster City, CA) model 432 synthesizer. Fluorenyl-methoxycarbonyl (Fmoc)-labelled amino acids were coupled as their hydroxybenzotriazole active esters following the manufacturer's standard protocols. The peptide was cleaved from the resin using trifluoroacetic acid/water/ethanedithol/thioanisol (900/30/30/40, by vol) and purified to near homogeneity by reversed-phase HPLC on a 2.5×25 cm Vydac 218TP1022 (C-18) column (Separations Group, Hesperia, CA). The identity of the peptide was confirmed by amino acid composition analysis and electrospray mass spectrometry (observed mass 2344.9; calculated mass 2345.2). All other peptides and reagents were supplied by Sigma Chemical, St Louis, MO).

2.2. Experimental animals

Ball pythons (*P. regius*) of both sexes (weight range 550–900 g) were obtained from a local animal supplier and transported to Aarhus University where they were kept in a vivarium (150×60×60 cm) at a daily photoperiod of 12/12 h light and darkness. The vivarium contained a heating lamp that provided a temperature gradient between 25 and 35 °C. The snakes appeared healthy and had free access to water but food had been withheld for at least one week before the experiments. All experiments were carried out by authorized investigators according to Danish Federal Regulations (protocol No. J1999/561-231).

2.3. Surgery and instrumentation

Snakes were anesthetized by an intramuscular injection of 30 mg kg^{-1} pentobarbital (Mebumal, Sygehusapotkerne, Denmark). All reflexes disappeared within 20 min and the animals were then placed in a prone position so that they could be traecheostemised for artificial ventilation at 4 breaths min^{-1} and a tidal volume of 50 ml kg^{-1} using a Harvard Apparatus mechanical ventilator. A 5 cm ventral incision was made cranial to the heart and a PE50 catheter containing heparinized saline 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 for measurements

Table 1
Effect of saline, SNP and L-NAME on hemodynamic parameters in the anesthetized python, *Python regius*

	P_{sys}(kPa)	Q_{sys} (ml min^{-1} kg^{-1})	G_{sys} (ml kPa^{-1} min^{-1} kg^{-1})	P_{pul} (kPa)	Q_{pul} (ml min^{-1} kg^{-1})	G_{pul} (ml kPa^{-1} min^{-1} kg^{-1})	f_H (min^{-1})
Pre-injection	6.8±0.8	40.9±7.9	6.0±0.6	1.2±0.2	29.0±5.2	23.9±1.9	37.8±3.0
Saline	6.8±0.8	41.6±7.8	6.1±0.6	1.2±0.2	29.5±4.9	24.1±1.9	37.6±2.9
Pre-injection	5.8±0.5	35.7±8.1	6.0±1.0	1.3±0.3	20.0±3.3	16.5±2.4	33.4±2.7
SNP	2.5±0.3*	40.6±10.9	14.9±3.3*	1.1±0.2	24.9±3.8*	23.4±2.2*	35.6±2.9*
Pre-injection	6.0±0.4	41.7±9.8	6.2±1.5	1.3±0.3	21.7±4.5	16.7±1.6	30.8±2.8
L-NAME	8.1±0.5*	37.7±10.1	4.1±1.3*	1.5±0.3	22.1±5.5	14.7±1.9	33.6±3.3

Values are means±SE. P_{sys}, mean systemic blood pressure; Q_{sys}, systemic blood flow; G_{sys}, systemic conductance; P_{pul}, mean pulmonary blood pressure; Q_{pul}, pulmonary blood flow; G_{pul}, pulmonary conductance; f_H, heart rate; SNP, sodium nitroprusside; L-NAME, L-nitroarginine methyl ester.

* Denotes a significant difference between values before and after administration ($P<0.05$).

of pulmonary arterial blood pressure. The left pulmonary, which is much smaller than the right pulmonary artery and carries less than a quarter of the total pulmonary blood flow (unpublished observations) was occlusively cannulated with PE50 containing heparinized saline. The catheters were connected to Baxter Edward (model PX600, Irvine, CA) 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 and were calibrated daily against a static water column.

For measurements of blood flows, 2S or 2R transit-time ultrasonic blood flow probes (Transonic System, Inc., NY) were placed around the left aortic arch (LAo) and the right pulmonary artery. 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) at 50 Hz.

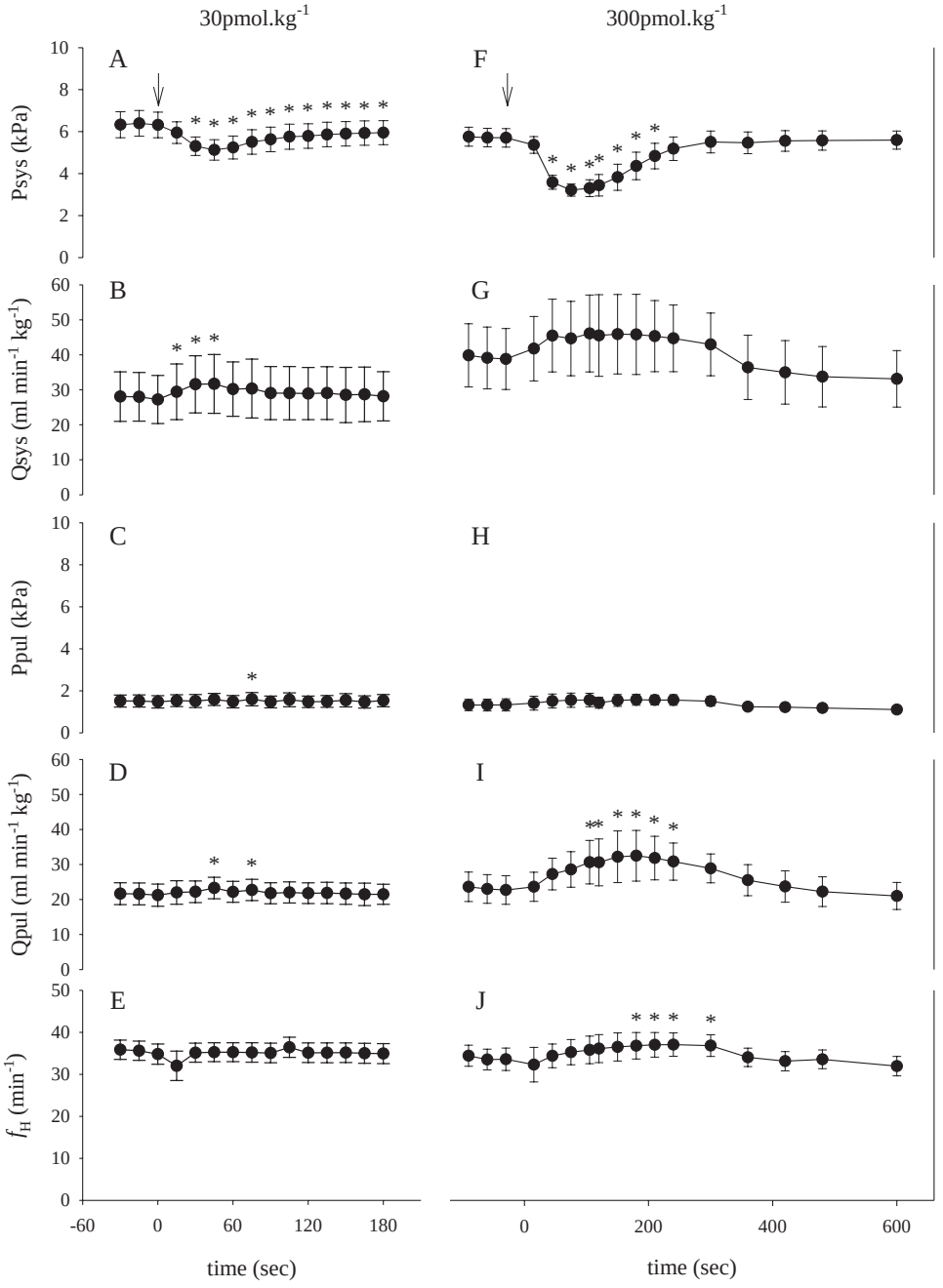

Fig. 1. Effects of a bolus intra-arterial injection of (A–E) 30 pmol kg^{-1} and (F–J) 300 pmol kg^{-1} python NPγ on hemodynamic parameters (P_{sys}, mean systemic arterial blood pressure; Q_{sys}, systemic blood flow; P_{pul}, mean pulmonary arterial blood pressure; Q_{pul}, pulmonary blood flow; f_H, heart rate) as a function of time. Data points show means±SE for 7 independent experiments. *Significantly different ($P<0.05$) from the corresponding values immediately before injection of peptide (denoted by ↓).

2.4. Calculation of blood flows, stroke volume and vascular conductances

Because the left pulmonary artery was occlusively cannulated, the measurement of blood flows in the right pulmonary artery represents total pulmonary blood flow (Q_{pul}). We did not measure blood flow in all systemic arteries, but placing probes around the left and right aortic arch of anaesthetized pythons indicate that systemic blood flow (Q_{sys}) can be estimated as $2.5 \times Q$LAo. Total cardiac output (Q_{tot}) was calculated as $Q_{sys}+Q_{pul}$. Heart rate (f_H) was calculated from the instantaneous blood flow trace from the left aortic arch and total stroke volume (VS_{tot}; pulmonary+systemic) was calculated as Q_{tot}/f_H. When baseline blood flow changes more than baseline blood pressure, which is the case in most in vivo situations, conductance provide a better index for comparing vascular tone than resistance [16,20]. Pulmonary and systemic conductance (G_{pul} and G_{sys}, respectively) were calculated from mean blood flow and mean blood pressure ($G_{pul}=Q_{pul}/$

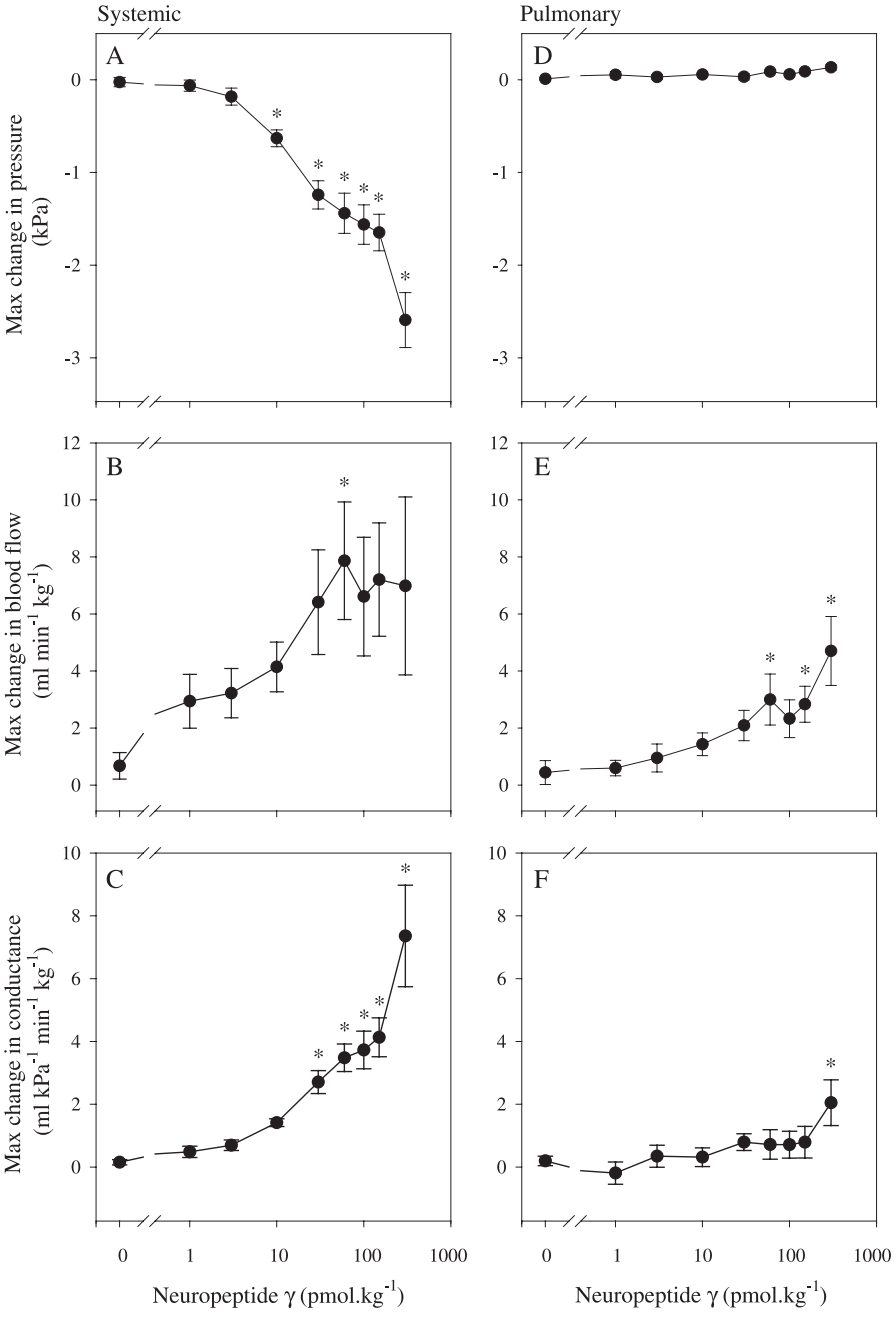

Fig. 2. Effects of a bolus intra-arterial injection of python NPγ on the maximum change in (A) P_{sys} and (D) P_{pul}; (B) Q_{sys} and (E) Q_{pul}; (C) G_{sys}, systemic vascular conductance and (F) G_{pul}, pulmonary vascular conductance as a function of the amount of peptide injected. Data points show means±SE for 7 independent experiments. *Denotes a significant difference ($P<0.05$) from pre-injection value.

P_{pul} and $G_{sys}=Q_{sys}/P_{sys}$) assuming that central venous blood pressures are negligible.

2.5. Experimental protocols

After instrumentation, all variables were recorded for a period of 45 min to permit animal recovery and to obtain basal values. After all hemodynamic parameters had stabilized; a 0.1 ml kg^{-1} injection of 0.9% (w/v) saline containing 0.5% (w/v) bovine serum (vehicle only) was given. All animals then received a series of bolus injections of increasing doses of NPγ as follows, 1, 3, 10, 30, 60, 100, 150, and 300 pmol kg^{-1}. Hemodynamic variables were allowed to return to baseline levels between each injection. A stock solution of peptide (100 μM) was prepared in 0.01 M HCl, and serial dilutions to appropriate concentration were made using vehicle solution.

To investigate the mechanism of action of NPγ, the animals were treated with the nitric oxide donor sodium nitroprusside (SNP) (60 μg kg^{-1}) and effects upon hemodynamic parameters were recorded. Following this, NO production was blocked by administration of the inhibitor of nitric oxide synthesis, L-nitroarginine methyl ester (L-NAME; 150 mg kg^{-1}). Once hemodynamic parameters had stabilized, three bolus injections of NPγ (30, 60, and 90 pmol kg^{-1}) were made.

In a further series of experiments, four pythons (wt range 460–580 g), instrumented as previously described, received a series of bolus injections of increasing doses of the NK1 receptor-selective agonist [Sar^9,$Met(O_2)^{11}$]SP [10] as follows: 0.3, 1.0, 3.0, 10, 30, 100 pmol kg^{-1}. Following a 10 min rest period, the NK2 receptor-selective agonist [βAla^8]NKA-(4–10)-peptide [17] was administered in the following doses: 3, 10, 30, 100, 300, 1000 pmol kg^{-1}. Once hemodynamic variables had stabilized, two doses of NPγ were injected (30 and 60 pmol kg^{-1}). The snakes were then treated with the inhibitor of prostaglandin synthesis, indomethacin (10 mg kg^{-1}). After a period of 30 min, two injections of NPγ (30 and 60 pmol kg^{-1}) were given and hemodynamic effects were recorded.

2.6. Data analysis and statistics

All recordings of blood flows and pressures were analysed using acqKnowledge data analysis software (version 3.7.1., Biopac, Goleta, CA). Effects on hemodynamic variables were assessed by a one-way or two-way ANOVA for repeated measurements followed by an a posteriori multiple comparison procedure (Dunnett's and Tukey's tests, respectively) to identify values that were significantly different from control values. Effects of L-NAME, SNP, sham injections of saline, and indomethacin were assessed using paired *t*-tests. Differences were considered statistically significant at a 95% level of confidence ($P<0.05$). All data are presented as mean±SE.

3. Results

3.1. Cardiovascular effects of python NPγ

There were no effects on hemodynamic variables of administration of vehicle only (Table 1). Fig. 1 shows the effect of a bolus injection of 30 pmol kg^{-1} and 300 pmol kg^{-1} python NPγ as a function of time. Both high and low doses produced immediate (within 30 s) and significant falls

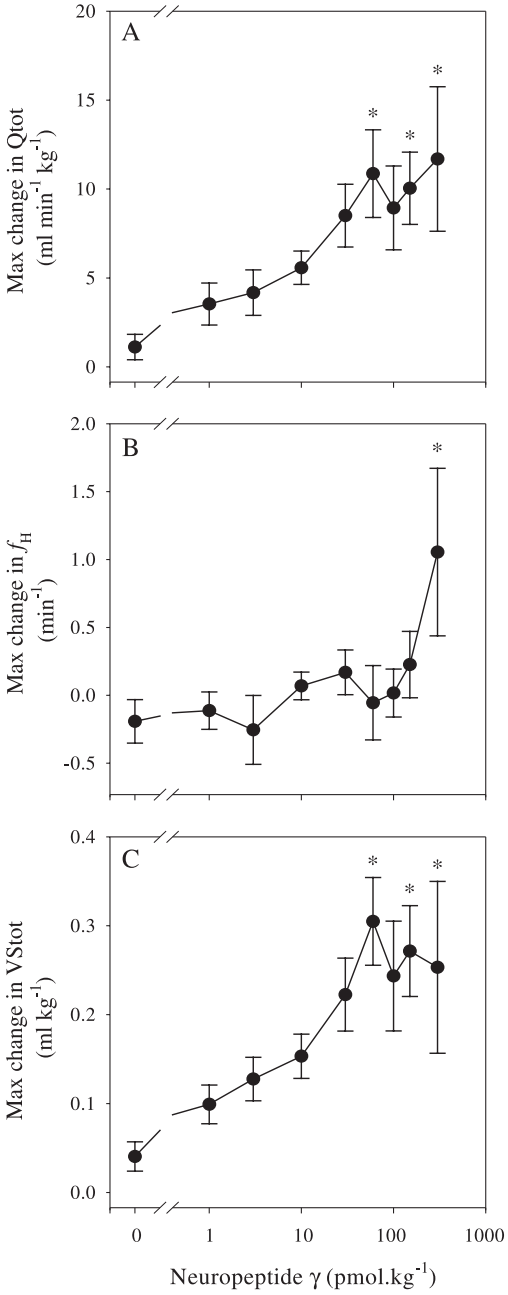

Fig. 3. Effects of a bolus intra-arterial injection of python NPγ on the maximum change in (A) Q_{tot}, total cardiac output; (B) f_H, heart rate; and (C) VS_{tot}, total stroke volume as a function of the amount of peptide injected. Data points show means±SE for 7 independent experiments. *Denotes a significant difference ($P<0.05$) from pre-injection value.

in systemic arterial blood pressure (P_{sys}) (panels A and F) that were not accompanied by corresponding changes in pulmonary pressure (P_{pul}) (panels C and H). The hypotensive response was accompanied by an increase in both systemic (Q_{sys}) (panels B and G) and pulmonary (Q_{pul}) (panels D and I) blood flows but no, or very small, increases in heart rate (f_H) (panels E and J). The dose-dependency of these effects is shown in Fig. 2. The threshold dose for a significant decrease in P_{sys} was 10 pmol kg^{-1} (panel A), whereas no change in P_{pul} was observed even at the highest dose (panel D). In view of the variability of the response, the increase in Q_{sys} was significant only after 60 pmol kg^{-1} (panel B) but the increase in Q_{pul} was significant after the 60, 150 and 300 pmol kg^{-1} injections (panel E). The marked dose-dependent increase in systemic vascular conductance (G_{sys}) (significant at doses above 30 pmol kg^{-1}) (panel C) was not mirrored by a corresponding increase in pulmonary conductance (G_{pul}) (panel F). As shown in Fig. 3, NPγ injection produced a dose-dependent increase in cardiac output (Q_{tot}) that was significant at 60, 150 and 300 pmol kg^{-1} (panel A). There was no change in f_H except for a small increase (approx 1 beat min^{-1}) at the highest dose (panel B). Total stroke volume (VS_{tot}) increased in a dose-dependent manner, although changes were significant only at doses above 60 pmol kg^{-1}.

3.2. Effects of receptor-selective agonists

Fig. 4 shows the effects of increasing doses of the NK1 receptor agonist [Sar^9,Met(O$_2$)^{11}]SP and the NK2 receptor-selective agonist [βAla^8]NKA-(4–10)-peptide on P_{sys} and P_{pul} (panels A and D), Q_{sys} and Q_{pul} (panels B and E), and G_{sys} and G_{pul} (panels C and F). The threshold doses of the NK1-selective agonist producing a significant fall in P_{sys}

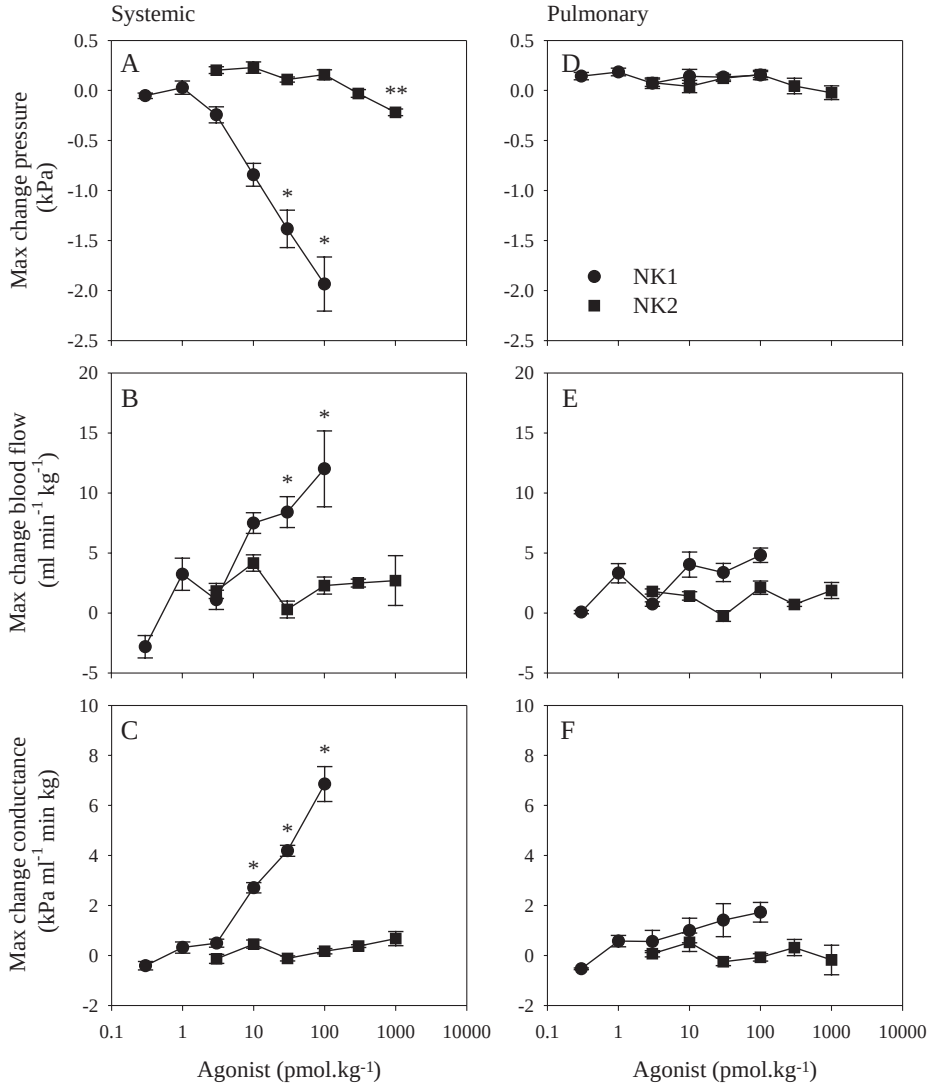

Fig. 4. Effects of bolus intra-arterial injection of the NK1 receptor agonist [Sar^9, Met(O$_2$)^{11})]SP (●–●) and the NK2 receptor-selective agonist ([βAla^8]NKA-(4–10)-peptide (■–■) on the maximum change in (A) P_{sys} and (D) P_{pul}; (B) Q_{sys} and (E) Q_{pul}; (C) G_{sys} and (F) G_{pul} as a function of the amount of peptide injected. Data points show means±SE for 4 independent experiments. *Denotes a significant difference (P<0.05) from 0.3 pmol kg^{-1} injection of the NK1 agonist. **Denotes a significant difference from 3 pmol kg^{-1} injection of the NK2 agonist.

and increase G_{sys} were 30 pmol kg^{-1} and 10 pmol kg^{-1}, respectively, whereas the NK2-selective agonist produced only a small fall in P_{sys} and had no effect on G_{sys} at a dose of 1000 pmol kg^{-1}. Consistent with the effects of python NPγ, the NK1-selective agonist did not produce significant effects upon P_{pul} or G_{pul} at doses up to 100 pmol kg^{-1}.

3.3. Effects of SNP and L-NAME

Administration of the NO donor, sodium nitroprusside (60 μg kg^{-1}) to the unstimulated animals produced significant falls in P_{sys} (57±4%; Table 1) and increase in G_{sys} (140±40%) but no change in Q_{sys}, Q_{tot}, and VS_{tot}. There was no change in P_{pul} but Q_{pul} and f_H increased slightly and G_{pul} also increased (48±9%). In contrast, administration of the inhibitor of NO formation, L-NAME (150 mg kg^{-1}) to the unstimulated animals produced significant increases in P_{sys} (37±9%; Table 1) and decrease in G_{sys} (32±9%) with no change in Q_{sys} or Q_{pul}, G_{pul}, Q_{tot} and f_H. There was a small but significant decrease in VS_{tot}.

The effect of pre-treatment with L-NAME upon the hemodynamic actions of NPγ is illustrated in Figs. 5 and 6. As L-NAME had marked effects on basal hemodynamic parameters, Figs 5 and 6 show relative changes before and after administration of the drug but the absolute magnitudes of these parameters are given in Table 2. The relative fall in P_{sys} (expressed as % of basal values) in response to 30 and 60 pmol kg^{-1} NPγ was significantly reduced after L-NAME

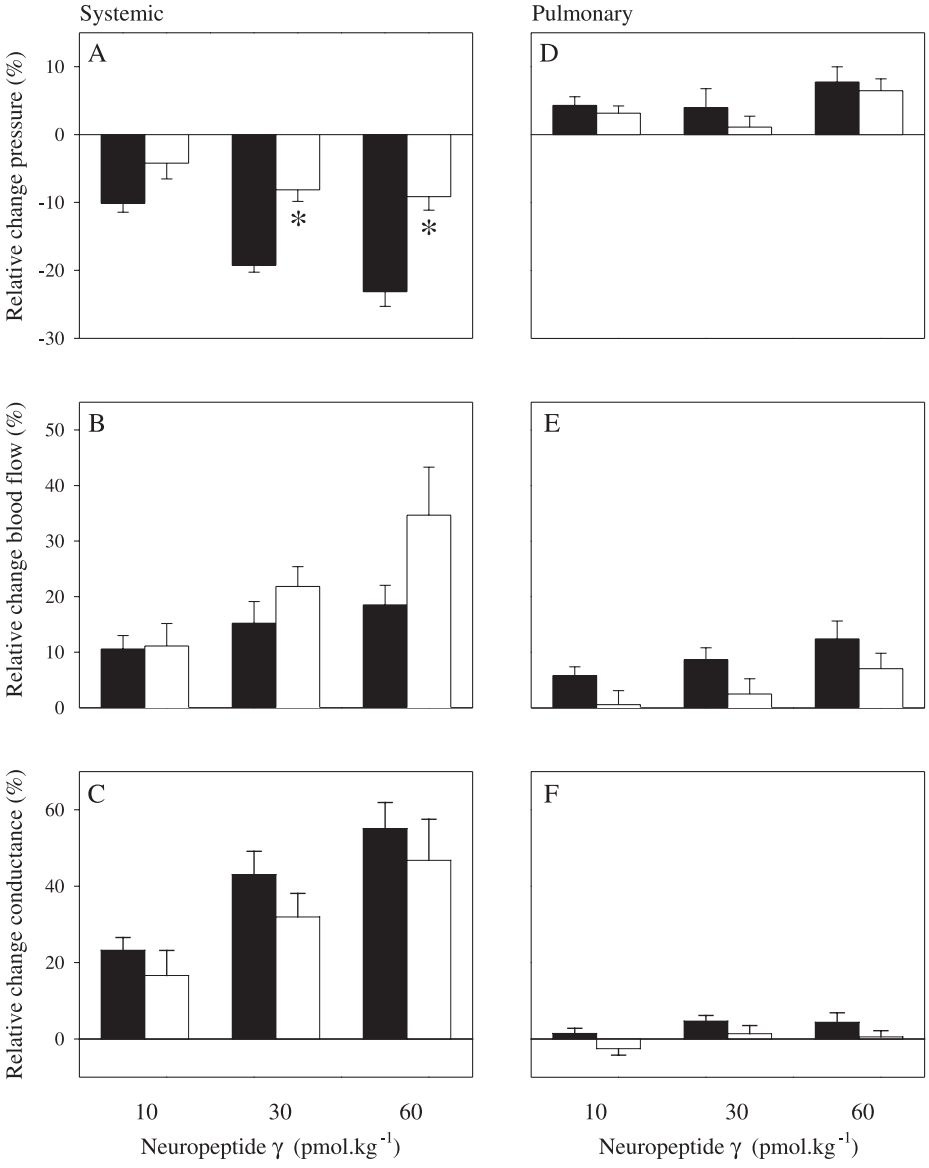

Fig. 5. Effects of bolus intra-arterial injections of 10, 30 and 60 pmol kg^{-1} python NPγ on relative changes in (A) P_{sys} and (D) P_{pul}; (B) Q_{sys} and (E) Q_{pul}; (C) G_{sys} and (F) G_{pul} before (filled bars) and after (open bars) treatment with L-NAME (150 mg kg^{-1}). Data points show means±SE for 6 independent experiments. *Denotes a significant difference from values before treatment (P<0.05).

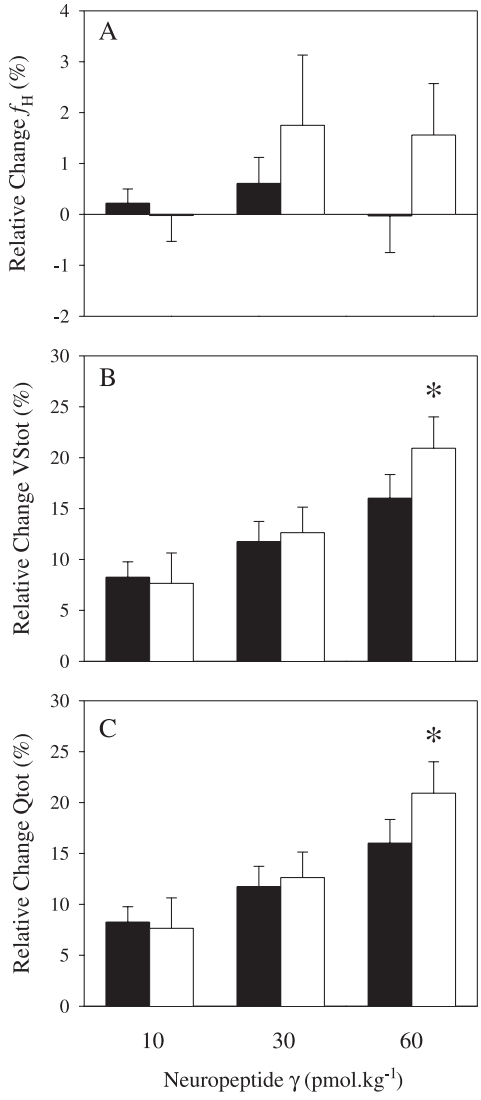

Fig. 6. Effects of bolus intra-arterial injections of 10, 30 and 60 pmol kg^{-1} python NPγ on relative changes in (A) Q_{tot}; (B) VS_{tot} and (C) f_H before (filled bars) and after (open bars) treatment with L-NAME (150 mg kg^{-1}). Data points show means±SE for 6 independent experiments. *Denotes a significant difference from values before treatment (P<0.05).

treatment but there was no change in the relative increase in either G_{sys} or G_{pul}. Similarly, there were no significant differences in the relative changes in Q_{sys} or Q_{pul}, Q_{tot}, VS_{tot}, and f_H in response to 10, 30 and 60 pmol kg^{-1} NPγ.

3.4. Effects of indomethacin

Intra-arterial injection of indomethacain (10 mg kg^{-1}) produced significant but transient increases in P_{sys} (96%), P_{pul} (69%), Q_{pul} (120%), Q_{tot} (103%) and f_H (61%) but no change in systemic or pulmonary vascular resistance. By 30 min, all parameters had returned to baseline value. As shown in Fig. 7, treatment with indomethacin did not significantly change the hemodynamic responses to 30 and 60 pmol kg^{-1} NPγ.

4. Discussion

Data of the present study have shown that the ball python (*P. regius*), as the case with the Burmese python (*Python molurus*) [25,26], exhibits complete pressure separation within the ventricle that allows for a low pulmonary blood pressure while maintaining mammalian-like pressures in the systemic circulation (Fig. 1). All hemodynamic variables were similar to those previously reported for anesthetized pythons [26,27]. With the exception of a higher heart rate, the hemodynamic status of the anesthetized animals in this study is similar to that of recovered, unanesthetized pythons [21,29].

4.1. Hemodynamic effects of NPγ

The hemodynamic effects of native NPγ in the systemic circulation of the python are qualitatively similar to those previously described for native SP in the same species [27]. Both peptides produced a pronounced and dose-dependent increase in vascular conductance resulting in a fall in arterial blood pressure concomitant with increases in blood flow and stroke volume. However, the threshold dose of SP producing a significant fall in systemic resistance (0.1 pmol kg^{-1}) was 300-fold less than the corresponding threshold dose of NPγ (30 pmol kg^{-1}).

In mammals, the effects of the tachykinins are mediated through interaction with three well characterized receptors (NK1, NK2, and NK3) that are differentiated pharmacologically by the rank order of potency of the naturally occurring agonists. NPγ, like NKA, is a preferred agonist for the mammalian NK2 receptor [9]. Intravenous injections of NPγ in the anesthetized guinea pig [32] and the anesthetized rabbit [33] also resulted in a fall in systemic arterial blood pressure. In both species, the NK1-selective agonist [Sar^9,Met(O$_2$)^{11}]SP was appreciably more potent than NPγ indicting that the hypotensive effect was mediated primarily through interaction with NK1 receptors. This conclusion was supported by the observation that the fall in pressure induced by NPγ was attenuated by the NK1 receptor selective antagonist, CP 96345 but unaffected by the NK2 receptor selective antagonist, SR 48968. Tachykinin receptors have not yet been characterized in a reptile so that the receptor classification of mammals does not strictly apply. However, our data suggest that the python cardiovascular system is associated with a receptor that resembles the mammalian NK1 receptor more closely than the NK2 receptor. The ligand binding properties of the NK1-like receptor in python are not identical to those of a mammalian NK1 receptor as the sensitivity of the python cardiovascular system to python SP ([Arg^3,Tyr^8]SP) was at least 100-fold greater than to [Sar^9,Met(0$_2$)^{11})]SP. The mammalian NK1 receptor selective agonist differs in structure from the endogenous python tachykinin at four sites [Arg^3→Lys, Tyr^8→Phe, Gly^9→Sar and Met^{11}→Met(0$_2$)^{11}]. Clearly, the effect of

Table 2
Effects of L-NAME on the hemodynamic responses to neuropeptide γ in the anesthetized python, *Python regius*

	P_{sys} (kPa)	Q_{sys} (ml min^{-1} kg^{-1})	G_{sys} (ml kPa^{-1} min^{-1} kg^{-1})	P_{pul} (kPa)	Q_{pul} (ml min^{-1} kg^{-1})	G_{pul} (ml kPa^{-1} min^{-1} kg^{-1})	f_H (min^{-1})
Before L-NAME							
Pre-injection	6.4±0.6	43.0±7.5	6.6±0.7	1.5±0.3	27.8±3.4	19.9±1.9	35.6±2.3
10 pmol NPγ	5.8±0.6*	47.2±7.7*	8.0±0.7*	1.6±0.3*	29.2±3.4*	20.2±2.0	35.6±2.3
Pre-injection	6.4±0.6	41.6±6.8	6.5±0.7	1.5±0.3	27.0±3.2	19.7±2.1	35.0±2.4
30 pmol NPγ	5.1±0.5*	48.0±7.9*	9.2±0.9*	1.5±0.3	29.1±3.2*	20.5±2.0*	35.2±2.3
Pre-injection	6.1±0.6	40.7±6.9	6.5±0.7	1.4±0.3	26.0±3.2	19.9±2.2	34.5±2.5
60 pmol NPγ	4.7±0.4*	48.5±8.5*	10.0±1.0*	1.5±0.3*	29.0±3.4*	20.6±2.2	34.4±2.4
After L-NAME							
Pre-injection	8.1±0.8	26.5±4.2	2.9±0.5	1.4±0.3	15.9±4.5	11.7±2.0	27.9±2.5
10 pmol NPγ	7.8±0.9	29.1±4.1*	3.4±0.7	1.4±0.3*	15.9±4.4	11.4±2.1	27.9±2.4
Pre-injection	7.9±0.5	33.7±9.4	3.6±1.1	1.7±0.4	18.3±4.7	11.1±1.4	31.0±3.8
30 pmol NPγ	7.3±0.5*	40.2±11.1*	4.8±1.5*	1.7±0.4	18.9±5.0	11.3±1.4	31.4±3.7
Pre-injection	8.0±0.6	30.8±8.0	3.2±0.8	1.6±0.4	17.0±3.7	11.0±1.3	30.4±3.8
60 pmol NPγ	7.3±0.5*	39.8±10.2*	4.7±1.3*	1.7±0.4	18.3±4.1	11.0±1.3	30.8±3.7

Values are means±SE. P_{sys}, mean systemic blood pressure; Q_{sys}, systemic blood flow; G_{sys}, systemic conductance; P_{pul}, mean pulmonary blood pressure; Q_{pul}, pulmonary blood flow; G_{pul}, pulmonary conductance; f_H, heart rate.

* Denotes a significant difference between values before and after administration ($P<0.05$).

some or all of these substitutions is to reduce the affinity of the synthetic agonist for the python NK1-like receptor relative to the naturally occurring ligand.

Structure-activity studies have shown that amino acid substitutions and deletions in the NH_2-terminal region of the molecule have little effect upon the interaction with the NK2 receptor in vitro. For example, in the isolated hamster urinary bladder, NPγ was equipotent with NKA in competing with ^{125}I-NKA for binding sites in a crude membrane preparation, stimulating phosphatidylinositol turnover, and in contracting isolated smooth muscle [24]. Similarly, the N-acetylated fragments (3–21)NPγ, (5–21)NPγ, (7–21)NPγ, and (9–21)NPγ were equipotent with each other and with NKA for binding to NK1 receptor sites in rat submandibular gland and to NK2 receptor sites in the rat gastric fundus [4]. However, the metabolic clearance rate of NKA is extremely high whereas the half-life in the circulation of N-terminally extended forms such as NPγ is appreciably longer so that intra-arterial infusions of NPγ produce hypotensive and bronchoconstrictor responses that are of enhanced magnitude and duration compared with NKA [23,32,33]. Thus, it seems unlikely the reduced potency of python NPγ compared with python SP is a consequence of a faster rate of clearance from the circulation.

Vascular resistance in the pulmonary circulations of mammals is regulated by a range of endogenous agents including the tachykinins (reviewed in Ref. [12]). The lack of the effect of injections of even very high doses of NPγ on pulmonary vascular conductance and pulmonary arterial pressure suggests, as one explanation, that appropriate receptors are present only at very low concentrations on the pulmonary blood vessels of the python. The actions of tachykinins on pulmonary hemodynamics in mammals appear to be species dependent. The effects of NPγ on the pulmonary circulation of a mammal has not been reported but intralobular injections of SP into the cat produced only small falls in pulmonary arterial pressure under basal conditions, but significant and dose-dependent decreases in this parameter when lobar vascular resistance was increased with U-46619, a thromboxane A2 mimetic [18]. Similarly, when pulmonary vascular tone was increased in the anesthetized dog [2] and pig [1] by prostaglandin $F_{2\alpha}$, intravenous infusions of SP produced pulmonary vasodilatation. In contrast, activation of NK1 receptors by injection of the selective agonist [Sar^9,Met(O$_2$)^{11}]SP increased pulmonary arterial pressure in the rat and the effect was attenuated by the NK_1 receptor selective antagonist CP-96345 [5]. In the isolated perfused guinea pig lung, SP also produced marked increases in pulmonary arterial pressure that were mediated through constriction of postcapillary vessels [22]. Indirect evidence for a pulmonary vasoconstrictor action of SP in a reptile was obtained in the estuarine crocodile, *Crocodylus porosus* [3]. Injection of mammalian SP into this species produced a right-to-left cardiac shunt that was ascribed to constriction of the pulmonary vasculature. It has been shown in rats that hypoxia increases the sensitivity of the pulmonary vessels to peptides that activate the NK1 receptor [5] so that further studies are warranted to investigate the action of native tachykinins on pulmonary hemodynamics in the python under hypoxic conditions.

4.2. The role of nitric oxide

Administration of the nitric oxide donor, SNP elicited a marked increase in G_{sys} while G_{pul} was increased to a much lower extent. Conversely, administration of L-NAME to block constitutive NO synthesis led to a substantial decrease

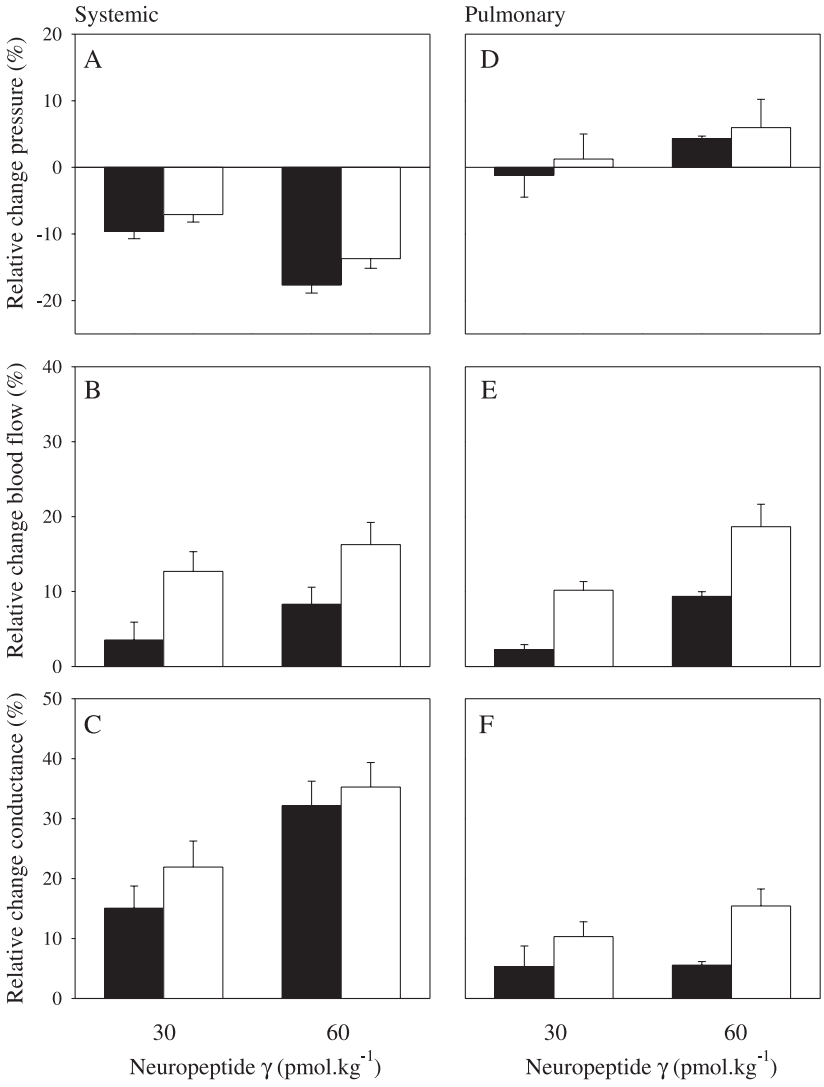

Fig. 7. Effects of bolus intra-arterial injections of 30 and 60 pmol kg^{-1} python NPγ on relative changes in (A) P_{sys} and (D) P_{pul}; (B) Q_{sys} and (E) Q_{pul}; (C) G_{sys} and (F) G_{pul} before (filled bars) and after (open bars) treatment with indomethacin (10 mg kg^{-1}). Data points show means±SE for 4 independent experiments. *Denotes a significant difference from values before treatment ($P<0.05$).

of G_{sys} while there was no significant effect on G_{pul}. Thus, it appears that NO is important for maintaining vascular tone in the systemic circulation while it plays a smaller role in the lung. Similar findings have been reported for turtles [8].

The involvement of nitric oxide in mediating the systemic vasodilation produced by the tachykinins in mammals has been firmly established [14]. In snakes, immunohistochemical studies have demonstrated the presence of nitric oxide synthase in the trigeminal ganglia of *Trimeresurus flavoviridis*, suggesting a role in the transmission of tactile and nociceptive sensations [19], and in neuronal structures in the gastrointestinal tract of *Thamnophis sirtalis* suggesting an inhibitory role on motility [15]. The present study provides evidence that, while nitric oxide is important in regulating systemic vascular tone of the python under basal conditions, it does not suggest that the vasodilatory action of NPγ in the systemic circulation is mediated through increased nitric oxide synthesis. Similarly, the inability of indomethacin, an inhibitor of prostaglandin synthesis, to attenuate the vasodilatator response to NPγ is consistent with the view that the peptide acts directly to relax vascular smooth muscle.

4.3. Perspectives

Our data and that of a previous study [27] demonstrate that tachykinins elicit a marked increase in systemic vascular conductance in the python. Given that tachykinins have substantial effects upon the gastrointestinal circulation in fish and reptiles [3,13], future studies will address the possible role of tachykinins in regulating blood flows to the digestive organs of *P. regius* during digestion of prey. Our study adds to the emerging picture of evolutionary changes in the role of tachykinins in regulating the cardiovascular system (reviewed in Ref. [6]). SP exhibits a potent vasodilatator action in the peripheral circulation of a bird

(chicken) and the endogenous SP-like peptide (bufokinin) induces a hypotensive response in *Bufo marinus*. In contrast, native SP and NKA increase both systemic and coelic resistances and cause hypertension in the rainbow trout, *Oncorhynchus mykiss* that is associated with bradycardia and a decrease in cardiac output. Bowfin SP-related peptide exerts a similar cardiovascular response in the bowfin *Amia calva*. High doses (10–50 nmol kg^{-1}) of dogfish SP produce a slight pressor response in the dogfish, *Scyliorhinus canicula* (Elasmobranchii), while lower doses are without effect on blood pressure or heart rate. Reptiles occupy a uniquely important position in the transition from nontetrapods to the higher vertebrates so that the vasodilatatory role of tachykinins in the python indicates that the transition from vasoconstriction to a vasodilatatory action has occurred in all tetrapods. Moreover, our study shows, for the first time in an ectothermic vertebrate, that NPγ does not affect the pulmonary vasculature appreciably. The pulmonary vasculature is also rather insensitive to NO and it is possible that the evolution of structurally complex lungs in mammals is associated with more pronounced local control of pulmonary vascular conductance.

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