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Cardiovascular actions of python bradykinin and substance P in the anesthetized python, *Python regius*

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Wang, Tobias, Michael Axelsson, Jorgen Jensen, and J. Michael Conlon. Cardiovascular actions of python bradykinin and substance P in the anesthetized python, *Python regius. Am J Physiol Regulatory Integrative Comp Physiol* 279: R531–R538, 2000.—The cardiovascular actions of python bradykinin (BK) and substance P (SP) have been investigated in the anesthetized ball python, *Python regius*. Bolus intra-arterial injections of python BK (0.03–3 nmol/kg) produced concentration-dependent increases in arterial blood pressure, heart rate (HR), and cardiac output concomitant with small decreases in systemic resistance and stroke volume. Intra-arterial injection of 3 nmol/kg python BK produced a tenfold increase in circulating concentration of norepinephrine, but epinephrine levels did not change. BK-induced tachycardia was attenuated (>90%) by the β-adrenergic receptor antagonist sotalol, and the hypertensive response was attenuated (>70%) by the α-adrenergic receptor antagonist prazosin, indicating that effects of python BK are mediated at least in part by activation of the extensive network of adrenergic neurons present in vascular tissues. Bolus intra-arterial injections of python SP in the range 0.01–30 pmol/kg produced concentration-dependent decreases in arterial blood pressure and systemic peripheral resistance concomitant with increases in cardiac output and stroke volume but with only minor effects on HR. The data suggest that kinins play a physiologically important role in cardiovascular regulation in the python.

squamate; kinin; vasoactive peptide; norepinephrine; adrenergic receptors

THE KALLIKREIN-KININ SYSTEM in mammals involves the sequential action of a series of well-characterized proteolytic enzymes. Activation of factor XII (Hageman factor) in blood at the site of tissue injury or in vitro by contact with a charged surface results in activation of plasma prekallikrein and generation of bradykinin (BK) by the cleavage of high-molecular mass kininogen. BK is rapidly degraded, primarily in the pulmonary circulation, by the action of carboxypeptidase N (kininase I), angiotensin-converting enzyme (kininase II), and endopeptidase 24.11 (3). The blood of some reptiles appears to contain all the components of the kallikrein-kinin system present in mammals (7). For example, treatment of plasma from the alligator, *Alligator mississipiensis* (6), and from the red-eared turtle, *Pseudemys scripta* (10), with a charged surface (glass beads) in the presence of a kinase inhibitor generated a kinin that differs from mammalian BK by one amino acid substitution ($Ser^6 \rightarrow Thr$).

The existence of a kallikrein-kinin system in snakes has not been demonstrated unequivocally. When treated with trypsin, plasma from the viper, *Bothrops jararaca*, generated a peptide that had hypotensive effects in this snake and contracted the snake uterus but did not elicit biological effects in mammals (i.e., no contraction of the rat uterus or guinea pig ileum and no vasodilatation of the perfused dog hindleg) (1). It was proposed therefore that snakes may have developed their own kallikrein-kinin system that generates a structurally different kinin with different bioactivity (1). Consistent with this hypothesis, incubation of heat-denatured plasma of a relatively primitive snake of the Boidea family, the reticulated python, *Python reticulatus*, with trypsin generated a BK-related peptide that differed from mammalian BK by two substitutions ($Arg^1 \rightarrow Ala$ and $Ser^6 \rightarrow Thr$) (9). Similar treatment of plasma from the more derived colubrid snakes, the bullsnake (*Pituophis melanoleucus sayi*) and the coachwhip (*Masticophis flagellum*) produced [Val^1,Thr^6]-BK (19). However, treatment of plasma from these snakes with glass beads, under conditions that generated [Thr^6]-BK in turtle and alligator, did not produce a kinin, and so it is probable that a component analogous to mammalian factor XII is not present in snake blood (9, 19).

The tachykinins are a family of myotropic peptides that have been identified by immunohistochemical and radioimmunoassay techniques in nervous or neuroendocrine tissues from all classes of vertebrate including reptiles (reviewed in Ref. 16). Tachykinins share a common amino acid sequence at the COOH-terminus, represented by -Phe-X_{aa}-Gly-Leu-Met-NH_2, where X_{aa} is an aromatic or branched-chain aliphatic residue (21), and this region interacts with receptors in smooth

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muscle (24, 25). The mammalian tachykinins comprise substance P (SP), neurokinin A, neurokinin B, and the NH_2-terminally extended forms of neurokinin A, neuropeptide K, and neuropeptide-γ (5). The primary structures of the tachykinins have been relatively poorly conserved during vertebrate evolution, and only the amino acid residues important for receptor binding are identical across species (reviewed in Ref. 33). In the case of reptiles, the identical SP from the Burmese python, *Python molurus* (13), and the desert tortoise, *Gopherus agassizii* (31), shows two amino acid substitutions ($Lys^3 \rightarrow$ Arg and $Phe^8 \rightarrow$ Tyr) compared with mammals, whereas SP from the alligator, *Alligator mississipiensis*, contains only the substitution ($Lys^3 \rightarrow$ Arg) (32).

The biological actions of native kinins in snakes are unknown. Elucidation of the primary structure of python BK and SP has permitted an investigation of the biological activities of these peptides in a snake with a view to gaining insight into their physiological roles. The objective of the present study therefore was to study the cardiovascular effects of synthetic replicates of python BK and SP in the anesthetized ball python, *Python regius*.

MATERIALS AND METHODS

Experimental animals. All experimental procedures were approved by University of Göteborg Ethical Committee (permit number 101/99). Ball pythons (*Python regius*) of both sexes, weighing between 650 and 900 g, were obtained from a local animal supplier and housed collectively in a vivarium (150 × 60 × 60 cm) with free access to water and under a 12:12-h light-dark photoperiod. The vivarium was equipped with a heating lamp, thus providing a temperature gradient between 25 and 35°C that allowed for behavioral thermoregulation. All animals appeared healthy and fasted for more than 6 wk before experiments.

Anesthesia, surgery, and hemodynamic measurements. The snakes were anesthetized by an intraperitoneal injection of 15–20 mg/kg pentobarbital sodium. This dose was generally sufficient to keep the snakes fully anesthetized for the duration of the experiment, but in one snake it was necessary to administer an additional dose of 10 mg/kg. When the snakes did not exhibit ciliary responses they were placed in a prone position and the trachea was exposed ~5–10 cm from the snout. The trachea was cannulated with tubing of suitable diameter for artificial ventilation at a rate similar to ventilation in resting and undisturbed python (14 ml/breath; 1.3 breaths/min) (22), using a Braun Melsungen type 874052 (Melsungen, Germany) ventilator.

To access the systemic arteries a 5–7 cm ventral incision was made immediately cranial to the heart, and any bleeding from small superficial vessels was stopped by cauterization. A P-50 (Clay Adams) catheter filled with heparinized saline (0.9% wt/vol) was occlusively inserted into the cranial vertebral artery and forwarded close to the junction with the right aortic arch. The catheters were connected to a DPT-6100 (Peter von Berg, Germany) pressure transducer that was calibrated daily against a static water column. For measurements of blood flows 1.0- to 1.5-cm sections of the left aortic arch and right aortic arch were freed from connective tissue, and 2S transit-time ultrasonic blood flow probes (Transonic System, Ithaca, NY) were placed around each vessel. Acoustical gel was infused around the blood flow probes to enhance the signal. The flow probe on the right aortic arch was placed caudally to the point where the carotid artery branches.

Data recording. The pressure transducer was connected to Grass polygraph (model 7G) recorder for appropriate amplification and filtering. The blood flow probes were connected to a transonic dual-channel blood flowmeter (T206) for measurements of mean blood flows; the blood flowmeter was in turn connected to the Grass polygraph. Heart rate (HR) was derived from the pulsatile flow in the right or left aortic arches using a Grass tachograph (model 7P44D). All the signals were fed into a PC computer for on-line data acquisition using Labview 5.1 (National Instrument, Austin, TX).

Calculation of systemic blood flow and systemic vascular resistance. Systemic blood flow (Q_{sys}) was calculated as the sum of the recorded blood flows in the left and right aortic arches, respectively. Because we placed the blood flow probe after the carotid artery branched from the right aortic, our measurements of blood flow in the right aortic arch and hence the calculated Q_{sys}, represents a slight underestimation. Systemic resistance (R_{sys}) was calculated as the ratio of mean blood pressure to Q_{sys} ($R_{sys} = P/Q_{sys}$) under the assumption that both atrial pressures are zero. Instantaneous HR was recorded on the basis of systemic blood pressure, and systemic stroke volume was calculated as Q_{sys}/HR.

Peptide synthesis. Python BK (Ala-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg) and python SP (Arg-Pro-Arg-Pro-Gln-Gln-Phe-Tyr-Gly-Leu-Met-NH_2) were synthesized by solid-phase methodology on a 0.025-mmol scale using an Applied Biosystems model 432 synthesizer. Fluorenylmethoxycarbonyl-labeled amino acids were coupled as their hydroxybenzotriazole-active esters following the manufacturer's standard protocols. After cleavage from the resin, the peptides were purified to near homogeneity by reversed-phase high-performance liquid chromatography (HPLC). Their identities were confirmed by automated Edman degradation and electrospray mass spectrometry (python BK, observed mass 988.5 Da, calculated mass 988.5 Da; python SP, observed mass 1,390.8 Da, calculated mass 1,390.7 Da). Turtle and/or alligator BK (Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg) was synthesized and characterized as previously described (10).

Experimental protocol. All experiments were conducted at 25°C. When the snakes were instrumented for measurements, blood flows and pressures were allowed to stabilize for ~60 min. Python BK and SP, dissolved in 0.9% saline containing 0.1% bovine serum albumin, were injected through the arterial catheter as a 0.1 ml/kg bolus followed by an injection of 0.2 ml/kg physiological saline to rinse the catheters. Hemodynamic variables were allowed to return to baseline levels between each injection, and there were no hemodynamic effects after injection of vehicle only ($n = 9$). The peptide injections invariably commenced with the lowest concentrations, and the series of python BK and SP injections were performed in random order. The following doses were administered: python SP 0.01, 0.1, 0.3, 1.0, 3.0, 10, and 30 pmol/kg; python BK 0.03, 0.1, 0.3, 1.0, and 3.0 nmol/kg. In six snakes, two doses of turtle and/or alligator BK (0.1 and 1.0 nmol/kg) were injected immediately before or after the same dose of python BK.

To investigate the mechanism that underlies the hypertensive response to BK, the effects of treating the snakes with β-adrenergic receptor antagonist sotalol and the α-adrenergic receptor antagonist prazosin were studied. In five snakes, the following drugs were injected through the arterial catheter in the following order: epinephrine (1 nmol/kg), sotalol (5.4 mg/kg), BK (3.0 nmol/kg), epinephrine (1 nmol/kg). In five snakes, the following drugs were injected in the following order: epinephrine (1 nmol/kg), prazosin (0.1 mg/

kg), BK (3.0 nmol/kg), epinephrine (1 nmol/kg). The injections of epinephrine were performed to assess the effectiveness of adrenergic blockade, and sotalol and prazosin were allowed to take effect for 20 min before subsequent injections were performed. Each injection was preceded by a control period, which was used as a baseline to calculate the changes in hemodynamic variables.

In a further series of experiments blood samples (1 ml) were taken from anesthetized animals ($n = 5$) via the arterial catheter following injection of python BK (3 nmol/kg) at a time when the pressor response was at a maximum. Plasma samples (0.5 ml) were obtained by centrifugation immediately after withdrawal of blood. To prevent oxidation, 10 μl of glutathione-EGTA (0.2 mol/l–0.2 mol/l in water) was added before storage at −70°C. Catecholamine concentrations were determined by HPLC analysis after extraction with alumina as described (4).

The snakes were killed by vascular injections of pentobarbital sodium after completion of the experimental protocol.

Data analysis and statistics. All recordings of blood flows and pressures were analyzed using AcqKnowledge data analysis software (version 3.23; Biopac, Goleta, CA). The effects of python BK and SP injections on hemodynamic variables were assessed by a one-way ANOVA for repeated measures. The effects of python BK and turtle BK were compared by a two-way ANOVA for repeated measures. In both cases, mean values that were significantly different from control values were identified by a subsequent Student-Newman-Keuls test. The effects of python BK injection on plasma catecholamine levels were assessed by a paired *t*-test. A limit for significance of $P < 0.05$ was applied, and data are presented as means ± SE.

RESULTS

Cardiovascular effects of python BK. Figure 1 shows the effects following a bolus injection of 0.3 nmol/kg python BK as a function of time (Fig. 1, *A-E*) and the dose-response relationships of the maximum hemodynamic changes at various concentrations (Fig. 1, *F-J*). Python BK caused an immediate and pronounced tachycardia. At the higher concentrations (0.3–3.0 nmol/kg), HR doubled relative to preinjection levels and remained elevated for up to 15 min. Stroke volume decreased initially but returned to preinjection values within 90 s following injection. Consequently, the massive tachycardia was associated with a large increase in Q_{sys} and, because R_{sys} decreased only slightly, the peptide caused a large increase in arterial blood pressure. The hemodynamic changes at the higher python BK concentrations were biphasic, such that Q_{sys} initially decreased slightly within the first 60 s after python BK injection with a simultaneous increase in R_{sys}. However, at all concentrations studied, R_{sys} significantly decreased at 120 s in a dose-dependent manner (Fig. 1*I*). The effects of 3 nmol/kg BK on any cardiovascular parameter were not significantly greater than the effects of 1 nmol/kg, indicating that the lower dose produced a near maximum response.

Effects of adrenergic blockers and levels of circulating catecholamines. The effects of adrenergic blockade on the hemodynamic responses to python BK injections are presented in Figs. 2 and 3. In untreated snakes, the bolus injections of epinephrine (1 nmol/kg) significantly elevated HR, systemic blood pressure, and Q_{sys}, whereas R_{sys} did not change. Injection of the β-adrenergic receptor antagonist sotalol did not affect blood pressure but caused a significant increase in R_{sys} and a small reduction in Q_{sys} from 9.6 ± 1.4 to 8.0 ± 2.5 ml · min^{-1} · kg^{-1} (not significant). Sotalol almost completely abolished the tachycardia following both python BK (3 nmol/kg) and epinephrine (1 nmol/kg) injections (Fig. 2*A*). Thus in untreated animals, python BK injection resulted in an increase in HR from 24.8 ± 3.3 to 42.2 ± 3.6 beats/min, whereas in sotalol-treated animals, HR did not increase significantly (from 19.9 ± 2.2 to 21.4 ± 2.3 beats/min). After sotalol, python BK did not reduce R_{sys}, whereas epinephrine injection led to a small increase in blood pressure and R_{sys} with no changes in Q_{sys}.

Treatment with the α-adrenergic receptor antagonist prazosin did not affect the tachycardia following python BK and epinephrine injections (Fig. 3*A*), but the effects on blood pressure were significantly reduced (Fig. 3*B*). Thus injection of python BK increased systemic blood pressure from 62.3 ± 9.6 to 106.1 ± 8.6 cmH_2O in untreated snakes, but this rise in pressure was decreased to a change from 54.0 ± 6.5 to 65.7 ± 12.5 cmH_2O after treatment with prazosin. In prazosin-treated animals, python BK did not affect peripheral resistance, whereas epinephrine injections reduced R_{sys}.

Bolus injections of python BK (3 nmol/kg) resulted in a significant tenfold elevation of norepinephrine plasma concentrations from a resting level of 0.24 ± 0.04 to 2.48 ± 0.41 ng/ml (Fig. 4). The preinjection concentrations of epinephrine (3.1 ± 2.2 ng/ml) were more than tenfold higher than the norepinephrine levels but did not rise significantly after injection of python BK (3.4 ± 2.0 ng/ml).

Comparison of the cardiovascular actions of python and turtle and/or alligator BK. The hemodynamic effects of bolus injections of turtle and/or alligator BK (0.1 and 1.0 nmol/kg) are compared with the corresponding effects of the injections of the same dose of python BK in Table 1. The effects of 0.1 nmol/kg turtle and/or alligator BK on HR and arterial blood pressure were significantly less that the corresponding effects of 0.1 nmol/kg of python BK but the effects of 1.0 nmol/kg injections of the peptides were not significantly different. There was no difference in the effects of python BK and turtle and/or alligator BK on cardiac output and R_{sys} at either dose tested.

Cardiovascular actions of python SP. The mean hemodynamic effects of a bolus intra-arterial injection of 3.0 pmol/kg python SP as a function of time are shown in Fig. 5, *A-E*, and the maximum hemodynamic changes following injections of the various concentrations of python SP are depicted in Fig. 5, *F-J*. Injection of python SP elicited a large reduction in systemic peripheral resistance (Fig. 5*I*) with an attendant decrease in systemic blood pressure (Fig. 5*H*), whereas Q_{sys} increased (Fig. 5*G*). There were no significant effects of python SP on HR, except for a slight increase after an injection of 10 pmol/kg (Fig. 5*F*), but the

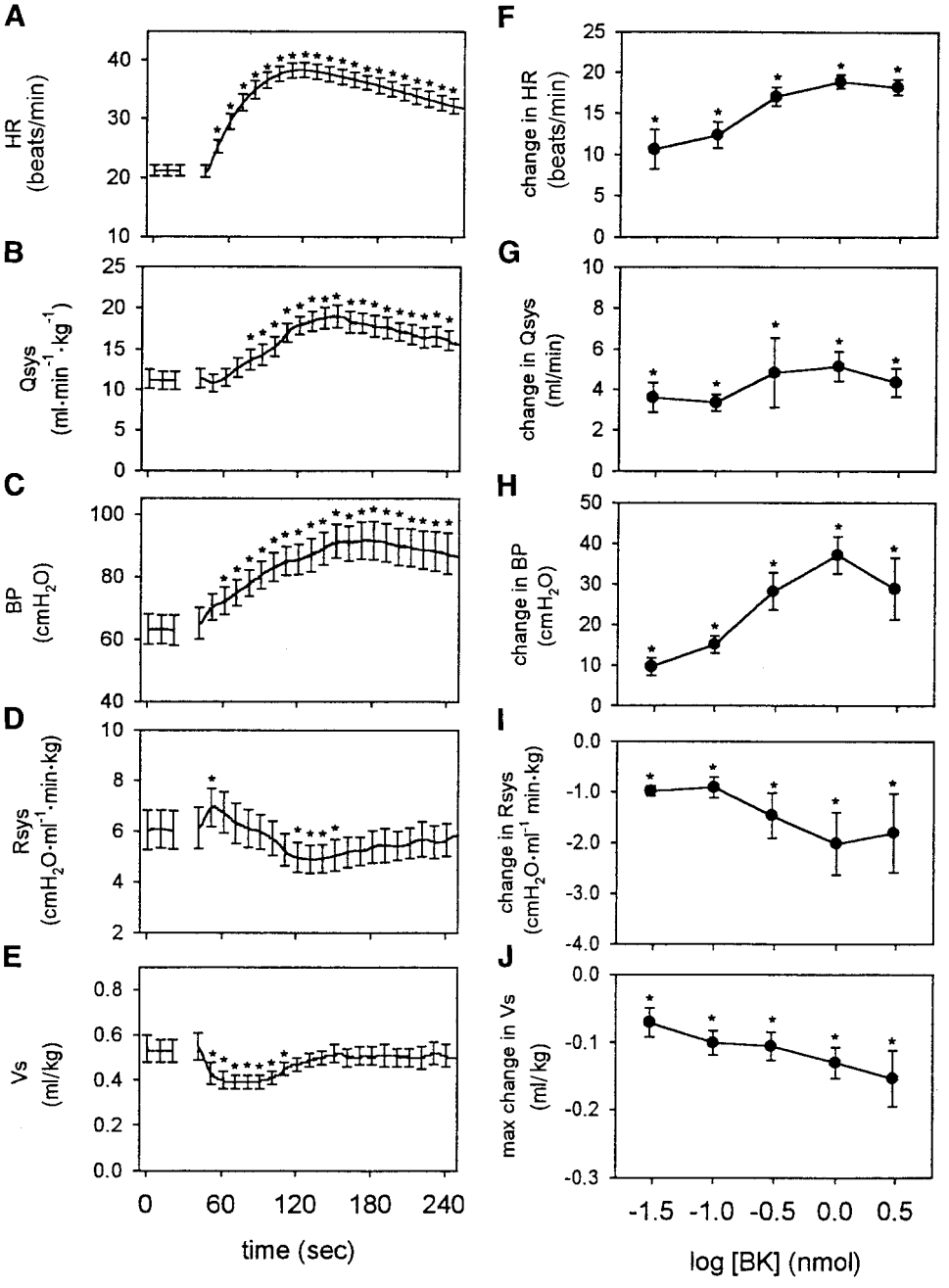

Fig. 1. Effects of a bolus intra-arterial injection of python bradykinin (BK, 3 nmol/kg) on hemodynamic parameters (HR, heart rate; Q_{sys}, systemic blood flow, BP, mean arterial blood pressure, R_{sys}, systemic vascular resistance; V_s, stroke volume) in the anesthetized python as a function of time (*A-E*) and the effects of the peptide as a function of the amount injected (*F-J*). Data points show means ± SE for 9 independent experiments. *Significantly different ($P < 0.05$) from the corresponding values following injection of vehicle only. In *A-E*, the peptide was administered at time +30 s.

peptide produced an increase in stroke volume (Fig. 5*J*). All hemodynamic effects, except HR, were augmented as the dose of python SP increased reaching a maximum response at a dose of 10 pmol/kg.

DISCUSSION

The cardiovascular actions of BK-related peptides differ appreciably among tetrapods (7). In mammals, vascular injection of BK rapidly reduces blood pressure by an arteriolar vasodilatation that arises from activation of prostaglandins and nitric oxide synthesis (15). At high doses and in unanesthetized animals, the hypotensive effect is followed by an increased blood pressure, HR, and renal vascular resistance caused by activation of nociceptive pathways and stimulation of afferent sympathetic fibers (29). In the chicken, bolus injections of ornithokinin ([Thr^6,Leu^8]-BK) produced a strong hypotensive response of short duration (<1 min) (18) and a similar response was observed following bolus injections of [Thr^6]-BK in anesthetized alligators (6). In contrast, bolus injections of [Thr^6]-BK produced no change in systemic blood pressure in the anesthetized turtles but an observed increase in blood flow in the left aortic arch indicated that this peptide reduced peripheral vascular resistance, as observed in alligators and mammals (10).

The present study was designed to investigate the direct effects of python BK and SP on cardiovascular

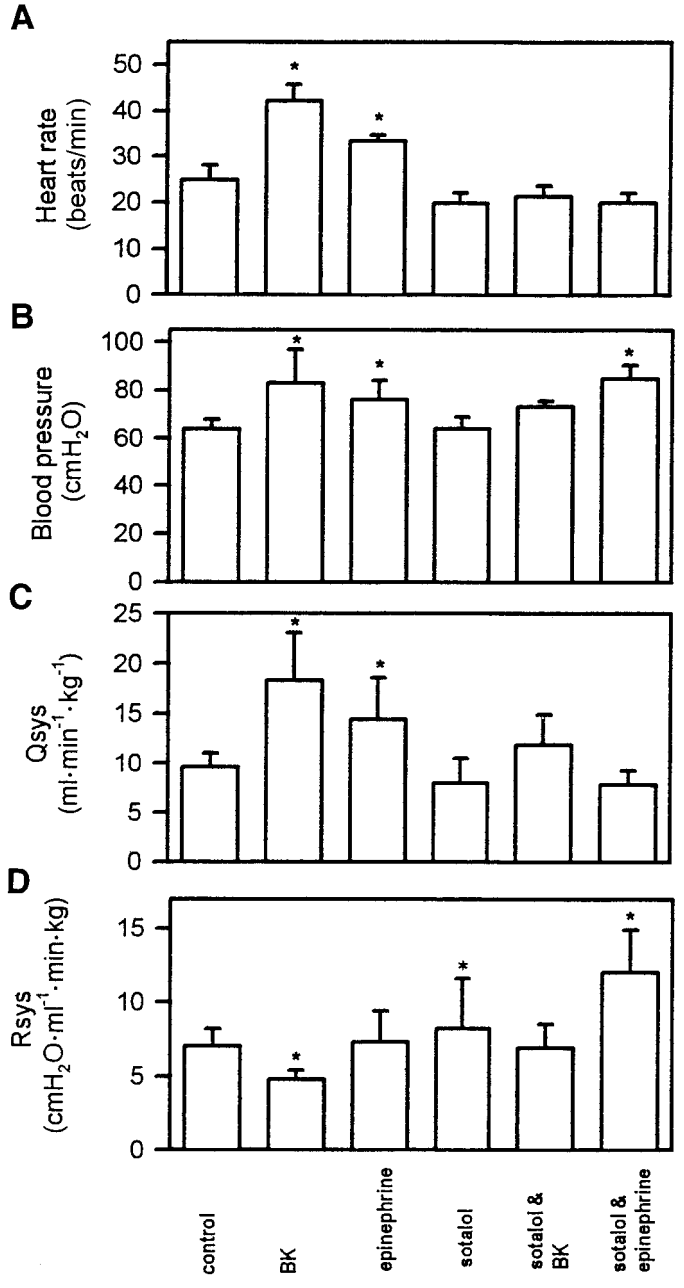

Fig. 2. Effect of bolus intra-arterial injections of python BK (3 nmol/kg) and epinephrine (1 nmol/kg) on hemodynanic parameters in the anesthetized python ($n = 5$) before and after treatment with sotalol. Control, effects following injection of vehicle only. *Significantly different ($P < 0.05$) from control values

parameters in pythons. We performed the experiments on phenobarbitone-anesthetized animals because barbiturates produce a general depression of central nervous system activity, including a reduction in respiratory drive and a dampening of cardiorespiratory reflexes. The use of relatively deep anesthesia, such that we did not observe behavioral or cardiovascular responses to stimuli such as tail pinching, means that effects arising from the activation of nociceptive pathways are probably not important. Furthermore, a study in anesthetized animals allows a direct comparison with data obtained with native BK peptides in the anesthetized turtle (10) and alligator (6). The mean values for arterial blood pressure and for concentrations of circulating catecholamines measured in the unstimulated state in the anesthetized python are comparable to those measured in the unrestrained resting snake, the black racer, *Coluber constrictor* (average daytime blood pressure 43 cmH_2O, HR 30–40 beats/min, norepinephrine 0.3 ± 0.1 ng/ml, epinephrine 1.1 ± 1.4 ng/ml) (28). However, it was shown that handling the snakes resulted in an increase in HR to

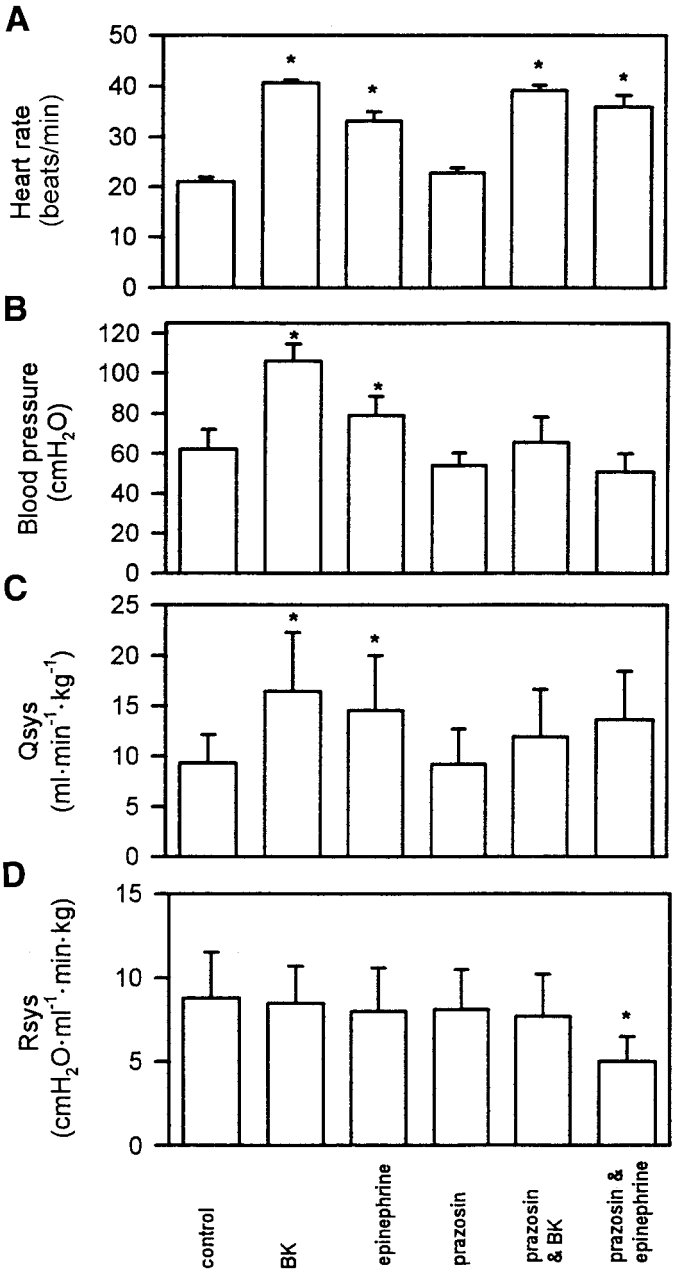

Fig. 3. Effect of bolus intra-arterial injections of python BK (3 nmol/kg) and epinephrine (1 nmol/kg) on hemodynanic parameters in the anesthetized python ($n = 5$) before and after treatment with prazosin. *Significantly different ($P < 0.05$) from control values following injection of vehicle only.

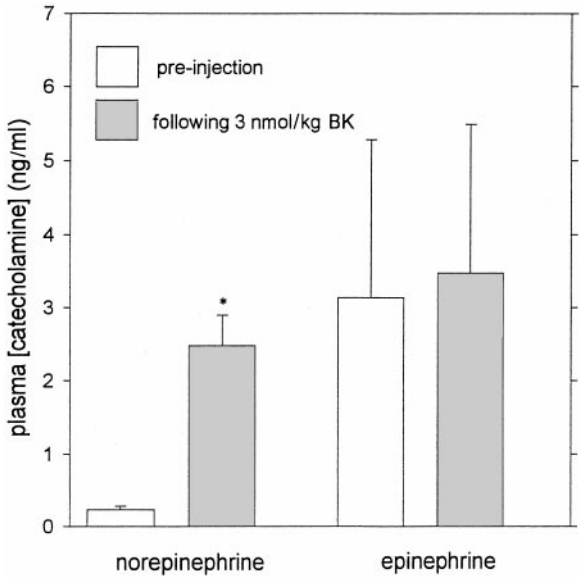

Fig. 4. Effect of a bolus intra-arterial injection of python BK (3 nmol/kg) on the concentrations of plasma catecholamines in the anesthetized python. Blood samples were taken at a time when the increase in arterial blood pressure was at a maximum. $*P < 0.05$ compared with preinjection concentrations.

near 100 beats/min and 51- and 26-fold increases in plasma epinephrine and norepinephrine, respectively (28). It is hoped that a future study will investigate the cardiovascular action of kinins in the unanesthetized python but clearly, in the light of such a marked stress response, this kind of study presents severe technical problems.

Our study has shown that bolus arterial injections of python BK ([Ala^1,Thr^6]-BK) into the anesthetized python increase arterial blood pressure, HR, and cardiac output in a concentration-dependent manner. These effects are clearly in marked contrast to those reported for the effects of [Thr^6]-BK in the anesthetized turtle (10) and alligator (6). However, as in these reptiles, BK reduces systemic vascular resistance in the python, and therefore the major difference between snakes and other reptiles is the pronounced tachycardia observed in the present study. Because sotalol treatment almost completely abolished the tachycardia following python BK injection, the effect appears to be mediated primarily by activation of cardiac β-adrenergic receptors. Similarly, the attenuation of the hypertensive response by prazosin indicates that this effect of python BK is mediated at least in part by activation of α-adrenergic receptors in the peripheral circulation. The involvement of catecholamines was confirmed by the significant rise in the concentration of circulating norepinephrine following administration of the peptide. Because the circulating levels of epinephrine did not change after BK administration, it appears plausible that the peptide primarily activates sympathetic neurons, as in mammals (29), rather than stimulating catecholamine release from the adrenal gland. Morphological studies, particularly in the colubrid snake, *Elaphe obseleta* (rat snake), have demonstrated an extensive adrenergic innervation of the arteries and veins with a particularly dense innervation of the arteries posterior to the heart (12, 14). Lillywhite and Donald (20) comment that this density is much greater than in any other class of reptile examined and postulate that this is indicative of the importance of adrenergic regulation of hemodynamic parameters in snakes. In rats, microinjections of mammalian BK into the anterior hypothalamic medial preoptic nuclei produce tachycardia that arises from activation of cardiac sympathetic nerves (13). It is not known whether BK is able to cross the blood-brain barrier in the python so that central effects of the peptide on stimulation of HR cannot be excluded.

Structure activity studies with mammalian BK have shown that the amino acid substitution (Ser^6 → Thr) in the peptide has little or no effect on its cardiovascular actions in the rat (23) and turtle (10). However, [Ala^1]-BK is only one-tenth as potent as BK in lowering blood pressure in the rat after intra-arterial injection of the peptides (23). Our observation that [Thr^6]-BK (1 nmol/kg) is equally effective as the same dose of [Ala^1,Thr^6]-BK in the python suggests that the anomalous cardiovascular actions of python BK in the python are not a consequence of the substitution (Arg^1 → Ala) in the peptide. It follows therefore that the ligand-binding properties of the BK receptor(s) in python tissues are appreciably different from the mammalian BK receptors.

The ability of SP to produce hypotension in mammals was one of the properties leading to its identification (30). In addition to the systemic vasodilatation that is mediated at least in part through release of nitric oxide (24), administration of SP to mammals and

Table 1. *Hemodynamic effects of bolus injections of python and turtle bradykinins in the anesthetized ball python (Python regius)*

	HR (max change), beats/min	P_{sys} (max change), cmH_2O	Q_{sys} (max change), $ml \cdot min^{-1} \cdot kg^{-1}$	R_{sys} (max change), cmH_2O $ml^{-1} \cdot min \cdot kg$
Python BK (0.1 nmol/kg)	12.5 ± 1.4	14.6 ± 4.9	4.6 ± 0.8	−0.90 ± 0.27
Turtle BK (0.1 nmol/kg)	8.7 ± 1.9*	7.2 ± 5.1*	4.1 ± 0.9	−1.24 ± 0.34
Python BK (1.0 nmol/kg)	18.3 ± 1.4	36.5 ± 5.6	9.4 ± 0.4	−1.74 ± 0.53
Turtle BK (1.0 nmol/kg)	18.7 ± 1.8	31.0 ± 4.2	8.7 ± 0.4	−1.92 ± 0.64

Values are means ± SE for 6 independent experiments. Q_{sys}, systemic blood flow; HR, heart rate; P_{sys}, mean systemic blood pressure; R_{sys}, systemic vascular resistance; BK, bradykinin. *Significantly different ($P < 0.05$) from the effects of injection with the same dose of python BK.

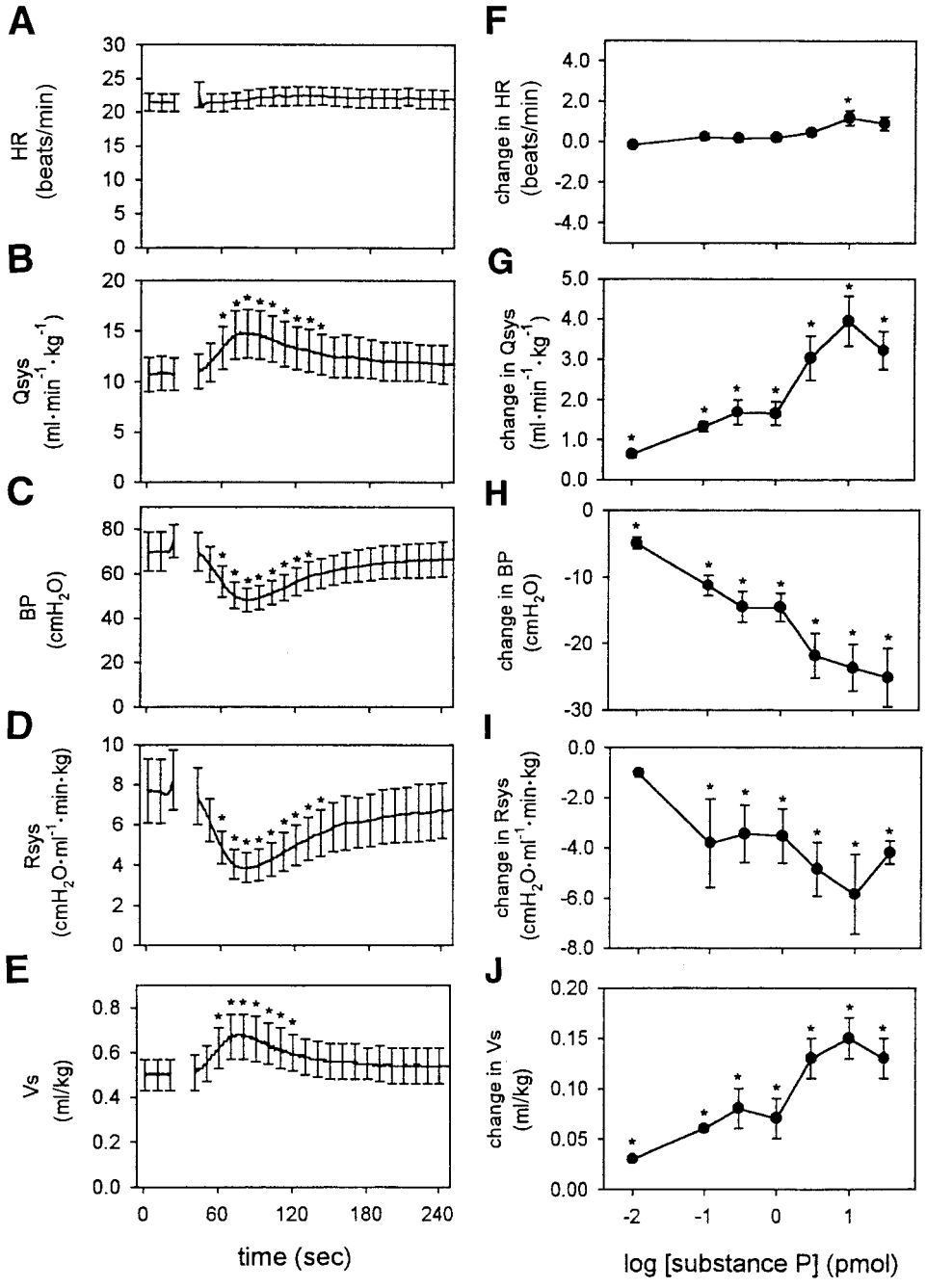

Fig. 5. Effects of a bolus intra-arterial injection of python substance P (3 pmol/kg) on hemodynamic parameters in the anesthetized python as a function of time (*A-E*) and the effects of the peptide as a function of the amount injected (*F-J*). Data points show means ± SE for 9 independent experiments. *Significantly different ($P < 0.05$) from the corresponding values following injection of vehicle only. In *A-E*, the peptide was administered at time +30 s.

crocodilians increases cardiac output and in particular elevates blood flow to the intestine (17). In the chicken (27) and the toad, *Bufo marinus* (11), SP also causes a transient hypotension. In contrast to the anomalous cardiovascular responses to python BK, bolus intra-arterial injections of python SP in the python had similar effects to those of mammalian SP in mammals that is a concentration-dependent decrease in arterial blood pressure and R_{sys} and an increase in cardiac output. In our experiments, there was only a minute increase in HR during the hypotensive phase, which is consistent with the absence of baroresponses in the phenobarbitone-anesthetized animals. Python SP contains conservative amino acid substitutions at positions 3 (Lys → Arg) and 8 (Phe → Tyr) compared with mammalian SP. However, a structure-activity study, in which each residue in the peptide was replaced by alanine, demonstrated that amino acids at these positions are not involved in the interaction with the SP receptor in vascular tissue of the rabbit (26). Unfortunately, sufficient animals were not available to study the mechanism of action of python SP in the python.

Perspectives

The sensitivity of the cardiovascular system of the python to BK (threshold dose 30 pmol/kg) and SP (threshold dose 10 fmol/kg) suggests that these pep-

tides may have a physiologically important role in the regulation of hemodynamic parameters in this snake. The circulating concentrations of BK in healthy mammals, e.g., human are extremely low but rise in pathological conditions that involve an inflammatory response (2). We speculate that BK production may be important in the python in response to tissue injury. Local generation of BK could potently stimulate the discharge of sympathetic neurons, resulting in a redistribution of blood flow in the region of the damaged tissue.

Immunohistochemical studies have shown the sinus venosus, atria, and ventricle of *E. obsoleta* (14) are innervated by SP-containing neurons, and there is also an extensive and dense innervation by SP of both anterior and posterior arteries and veins where it is often colocalized with vasoactive intestinal peptide (12). We speculate therefore that release of SP from these neurons may have a functionally important role in maintaining adequate blood flow to vital anterior organs, particular when the python is adopting an upright posture during climbing (20).

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REFERENCES

1. **Abdalla FMF, Hiraichi E, Picarelli ZP, and Prezoto BC.** Kallikrein-kinin system in the plasma of the snake, *Bothrops jararaca*. *Br J Pharmacol* 98: 252–258, 1989.
2. **Balks HJ, Conlon JM, Creutzfeldt W, and Stockman F.** Circulating bradykinin-like immunoreactivity and the pentagastrin-induced carcinoid flush. *Clin Endocrinol (Oxf)* 28: 141–151, 1988.
3. **Bhoola KD, Figueroa CD, and Worthy K.** Bioregulation of kinins: kallikreins, kininogens and kininases. *Pharmacol Rev* 44: 1–80, 1992.
4. **Butler PJ, Axelsson M, Ehrenström F, Metcalfe JD, and Nilsson S.** Circulating catecholamines and swimming performance in the Atlantic cod, *Gadus morhua. J Exp Biol* 141: 377–387, 1989.
5. **Carter MS and Krause JE.** Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neuropeptide K, and neuropeptide γ. *J Neurosci* 10: 2203–2214, 1990.
6. **Comeau S, Lance VA, Hicks JW, and Conlon JM.** Purification and biological activity of alligator bradykinin. *Am J Physiol Regulatory Integrative Comp Physiol* 263: R400–R404, 1992.
7. **Conlon JM.** Bradykinin and its receptors in non-mammalian vertebrates. *Regul Pept* 79: 71–81, 1999.
8. **Conlon JM, Adrian TE, and Secor SM.** Tachykinins (substance P, neurokinin A and neuropeptide γ) and neurotensin from the intestine of the Burmese python, *Python molurus*. *Peptides 18*: 1505–1510, 1997.
9. **Conlon JM and Lance VA.** Generation of [Ala^1,Thr^6]bradykinin in the plasma of a snake, the reticulated python. *Gen Comp Endocrinol* 94: 273–278, 1994.
10. **Conlon JM, Smith DD, and Hicks JW.** Isolation and biological activity of a novel kinin ([Thr^6]bradykinin) from the turtle, *Pseudemys scripta*. *Endocrinology* 126: 985–991, 1990.
11. **Courtice GP, Burcher E, Carlo-Stella R, and Conlon JM.** Cardiovascular effects of amphibian and mammalian tachykinins in the toad, *Bufo marinus*. *Neuropeptides* 24: 171–176, 1993.
12. **Davies PJ and Donald JA.** Distribution and colocalization of neuropeptides in the large systemic arteries and veins of the ratsnake, *Elaphe obsoleta. Cell Tissue Res* 269: 495–504, 1992.
13. **Diz DI and Jacobowitz DM.** Cardiovascular effects of discrete intrahypothalamic and preoptic injections of bradykinin. *Brain Res Bull* 12: 409–417, 1984.
14. **Donald JA, O'Shea JE, and Lillywhite HB.** Somatostatin and the innervation of the heart of the ratsnake, *Elaphe obsoleta*. *Am J Physiol Regulatory Integrative Comp Physiol* 258: R1001–R1007, 1990.
15. **D'Orleans-Juste P, de Nucci G, and Vane JR.** Kinins act on B_1 or B_2 receptors to release conjointly endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells. *Br J Pharmacol* 96: 920–926, 1989.
16. **Jensen J.** Substance P and other tachykinins. In: *The Comparative Physiology of Regulatory Peptides*, edited by Holmgren S. New York: Chapman and Hall, 1989, p. 130–149.
17. **Kagstrom J, Olsson C, Axelsson M, and Franklin CE.** Peptidergic control of gastrointestinal blood flow in the estuarine crocodile, *Crocodylus porosus. Am J Physiol Regulatory Integrative Comp Physiol* 274: R1740–R1750, 1998.
18. **Kimura M, Sueyoshi T, Takada K, Tanaka K, Morita T, and Iwanaga S.** Isolation and characterization of ornithokininogen. *Eur J Biochem* 168: 493–501, 1987.
19. **Li Z, Secor SM, Lance VA, Masini M, Vallarino M, and Conlon JM.** Characterization of bradykinin-related peptides generated in the plasma of six Sarcopterygian species (African lungfish, amphiuma, coachwhip, bullsnake, Gila monster, and Gray's monitor). *Gen Comp Endocrinol* 112: 108–114, 1998.
20. **Lillywhite HB and Donald JA.** Neural regulation of arterial blood pressure in snakes. *Physiol Zool* 67: 1260–1283, 1994.
21. **Maggio JE and Mantyh PW.** History of tachykinin peptides. In: *The Tachykinin Receptors*, edited by Buck SH. Totowa, NJ: Humana, 1994, p. 1–21.
22. **Overgaard JM, Busk M, Hicks JW, Jensen FB, and Wang T.** Acid-base status and arterial oxygen transport following feeding in the snake, *Python molurus. Comp Biochem Physiol*. 124A: 359–365, 2000.
23. **Regoli D and Barabe J.** Pharmacology of bradykinin and related kinins. *Pharmacol Rev* 32: 1–46, 1980.
24. **Regoli D, Dion S, Rhaleb NE, Drapeau G, and D'Orleans-Juste P.** Vasoactive peptides and their receptors. *Blood Vessels* 27: 137–145, 1990.
25. **Regoli D, Drapeau G, Dion S, and D'Orléans-Juste P.** Receptors for substance P and related neurokinins. *Pharmacology* 38: 1–5, 1989.
26. **Regoli D, Escher E, and Mizrahi J.** Substance P-structure-activity studies and the development of antagonists. *Pharmacology* 28: 301–320, 1984.
27. **Sann H, Friedrich R, and Pierau FK.** Substance P and calcitonin gene-related peptide in the chicken skin: distribution and cardiovascular effects. *Neuropeptides* 30: 273–281, 1996.
28. **Stinner JN and Ely DL.** Blood pressure during routine activity, stress and feeding in black racer snakes (*Coluber constrictor*). *Am J Physiol Regulatory Integrative Comp Physiol* 264: R79–R84, 1993.
29. **Uchida Y and Murao S.** Bradykinin-induced excitation of afferent cardiac sympathetic fibers. *Jpn Heart J* 15: 84–91, 1974.
30. **Von Euler US and Gaddum JH.** An unidentified depressor substance in certain tissue extracts. *J Physiol (Lond)* 72: 74–87, 1931.
31. **Wang Y, Lance VA, Nielsen PF, and Conlon JM.** Neuroendocrine peptides (insulin, pancreatic polypeptide, NPY, galanin, somatostatin, substance P, neuropeptide γ) from the desert tortoise, *Gopherus agassizii. Peptides* 20: 713–722, 1999.
32. **Wang Y, O'Harte F, and Conlon JM.** Structural characterization of tachykinins (neuropeptide γ, neurokinin A, substance P) from a reptile, *Alligator mississipiensis*. *Gen Comp Endocrinol* 88: 277–286, 1992.
33. **Waugh D, Bondareva V, Rusakov Y, Bjenning C, Nielsen PF, and Conlon JM.** Tachykinins with unusual structural features from a urodele, the amphiuma, an elasmobranch, the hammerhead shark and an agnathan, the river lamprey. *Peptides* 16: 615–621, 1995.