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Cardiovascular actions of rattlesnake bradykinin ([Val[1],Thr[6]]bradykinin) in the anesthetized South American rattlesnake *Crotalus durissus terrificus*

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Galli, Gina L. J., Nini Skovgaard, Augusto S. Abe, Edwin W. Taylor, J. Michael Conlon, and Tobias Wang. Cardiovascular actions of rattlesnake bradykinin ([Val[1],Thr[6]]bradykinin) in the anesthetized South American rattlesnake *Crotalus durissus terrificus. Am J Physiol Regul Integr Comp Physiol* 288: R456–R465, 2005. First published October 21, 2004; doi:10.1152/ajpregu.00417.2004.—Incubation of heat-denatured plasma from the rattlesnake *Crotalus atrox* with trypsin generated a bradykinin (BK) that contained two amino acid substitutions (Arg[1] → Val and Ser[6] → Thr) compared with mammalian BK. Bolus intra-arterial injections of synthetic rattlesnake BK (0.01–10 nmol/kg) into the anesthetized rattlesnake, *Crotalus durissus terrificus*, produced a pronounced and concentration-dependent increase in systemic vascular conductance (Gsys). This caused a fall in systemic arterial blood pressure (Psys) and an increase in blood flow. Heart rate and stroke volume also increased. This primary response was followed by a significant rise in Psys and pronounced tachycardia (secondary response). Pretreatment with N^G-nitro-L-arginine methyl ester reduced the NK-induced systemic vasodilatation, indicating that the effect is mediated through increased NO synthesis. The tachycardia associated with the late primary and secondary response to BK was abolished with propranolol and the systemic vasodilatation produced in the primary phase was also significantly attenuated by pretreatment, indicating that the responses are caused, at least in part, by release of cathecholamines and subsequent stimulation of β-adrenergic receptors. In contrast, the pulmonary circulation was relatively unresponsive to BK.

reptile; vasoactive kinin; catecholamines; nitric oxide; adrenergic receptor

BRADYKININ (BK) is a peptide produced in the blood in response to tissue injury that exerts pronounced cardiovascular effects in all animals studied. These effects may contribute to regulation of local blood perfusion of damaged tissue and be involved in the paracrine regulation of organ perfusion (6). The effects of BK in mammals are mediated through the interaction with two well-characterized receptors, termed B1 and B2 (17). BK acts directly with receptors on smooth muscle to cause vasodilatation of the systemic vasculature. At higher doses, this response is rapidly followed by a rise in systemic pressure (Psys) above resting levels as heart rate and cardiac output increase (reviewed in 25). In reptiles, infusion of the species-specific BK consistently causes a systemic vasodilatation, but the changes in blood pressure and heart rate vary among species. In anesthetized alligators, *Alligator mississipiensis,* Psys decreased after injection of BK, but there was no secondary hypertensive response or increased heart rate (4). Turtles, *Trachemys scripta,* also exhibited a systemic vasodilatation after injection of BK, which was accompanied by an increased systemic blood flow (Qsys), while Psys remained constant. However, unlike mammals, turtles do not exhibit a tachycardia (8). In the python, *Python regius*, Psys increased after injection of python BK, despite a systemic vasodilatation, because of a pronounced tachycardia. In pythons, infusion of BK caused a 10-fold increase in circulating norepinephrine, and all cardiovascular responses could be completely blocked by pharmacological inhibition of adrenergic receptors (28). The effects of BK on the pulmonary circulation of reptiles have not been previously studied. However, the pulmonary vasculature in reptiles is generally unresponsive to humoral and local factors compared with the systemic circulation (9, 23, 24; also, Galli, Skovgaard, Abe, Taylor, Conlon, and Wang, unpublished observations).

Bradykinin is generated in the blood by the kallikrein-kinin system and involves sequential action of a series of proteolytic enzymes. Activation of mammalian factor XII (Hageman factor) at the site of tissue injury catalyzes plasma prekallikrein and generates BK by cleavage of high-molecular-mass kininogen (2). BK can also be produced from kininogens in vitro by incubation of plasma with a charged surface such as glass beads or by trypsinization (10). BK is rapidly degraded, primarily in the pulmonary circulation.

The blood of reptiles contains some, but not necessarily all, components of the kallikrein-kinin system present in mammals. For example, BK is not generated from the plasma of lizards and snakes through contact with a charged surface, which indicates their blood does not contain a component analogous to mammalian factor XII. BK has, nevertheless, been generated from the plasma of all major groups of reptiles, including several lizards and snakes, but the primary structure differs considerably (reviewed in 6).

In the present study, we describe the purification and structural characterization of BK from a representative of the highly evolved Viperidae family of snakes, the diamondback rattlesnake, *C. atrox*, from North America. We then investigate the effects of bolus intra-arterial injections of a synthetic replicate of rattlesnake BK on the systemic and pulmonary circulations of anesthetized specimens of the South American rattlesnake, *C. durissus terrificus*. *C. durissus terrificus* and *C. atrox* are

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relatively closely related within the genus of rattlesnakes (20). Given their close phylogenetic relationship and the observation that the structure of rattlesnake BK is identical to that of other derived snakes (15), the potential problems of characterizing the cardiovascular effects of BK from *Crotalus atrox* in *Crotalus durissus* are not likely to affect the validity of our study.

MATERIALS AND METHODS

Characterization and Synthesis of Rattlesnake BK

Generation of the kinin. A sample (11 ml) of heparinized plasma from a single specimen of the diamondback rattlesnake (*C. atrox*) was provided by Dr. Valentine A. Lance (Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, CA) and was stored at −70°C. Plasma was diluted 10-fold with 0.2% (vol/vol) acetic acid and heated on a boiling water bath for 30 min. After cooling to 25°C, pH was adjusted to 7.8 by addition of 0.5 M Tris·HCl. The denatured plasma was incubated with 1-tosylamide-2-phenylethylchloro-methyl ketone-treated trypsin (Sigma, St. Louis, MO) (6 mg) for 30 min at 37°C and centrifuged (1600 g for 20 min). Peptide material was isolated from the supernatant using Sep-Pak C_{18} cartridges as previously described (7). Bound material was eluted with 70% (vol/vol) acetonitrile/water and lyophilized.

Radioimmunoassay. BK-like immunoreactivity (BK-LI) was measured using antiserum BT4, as previously described (15). The antiserum was raised against mammalian BK in rabbits and the epitope in BK recognized by the antiserum involves contributions from residues 3, 5, 6, and 9.

Purification of the kinin. The trypsin-treated plasma extract, after partial purification on Sep-Pak cartridges, was redissolved in 1% (vol/vol) trifluoroacetic acid/water (3 ml) and chromatographed on a 90 × 1.6 cm column of Sephadex G-25 (Pharmacia Biotech, Uppsala, Sweden) equilibrated with 1 M acetic acid. The column was eluted at a flow rate of 24 ml/h, and fractions were collected. Absorbance was measured at 280 nm. Aliquots of the fractions (100 μl) were lyophilized and reconstituted in the same volume of assay buffer [0.05 M sodium phosphate, pH 7.4 containing 0.4% (wt/vol) BSA]. The concentration of BK-LI in the fractions was determined by radioimmunoassay. Fractions containing BK-LI were pooled and injected onto a 25 × 1-cm Vydac 218TP510 C_{18} reverse-phase HPLC column (Separations Group, Hesperia CA) equilibrated with 0.1% trifluoroacetic acid/water at a flow rate of 2 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 14% over 10 min and to 35% over 50 min using linear gradients. Absorbance was monitored at 214 and 280 nm and fractions (1 min) were collected. The fraction containing BK-LI was rechromatographed on a 25 × 0.46-cm Vydac 214TP54 (C_4) column equilibrated with acetonitrile/water/trifluoroacetic acid (10.5/89.4/0.1) at a flow rate of 1.5 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 38% over 40 min using a linear gradient. Rattlesnake BK was purified to near homogeneity by successive chromatographies on 25 × 0.46 cm Vydac 219TP54 (phenyl) and Vydac 218TP54 C_{18} columns. The concentration of acetonitrile in the eluting solvent was raised from 7 to 35% over 40 min, and the flow rate was 1.5 ml/min.

Structural analysis. The primary structure of the peptide was determined by automated Edman degradation using an Applied Biosystems

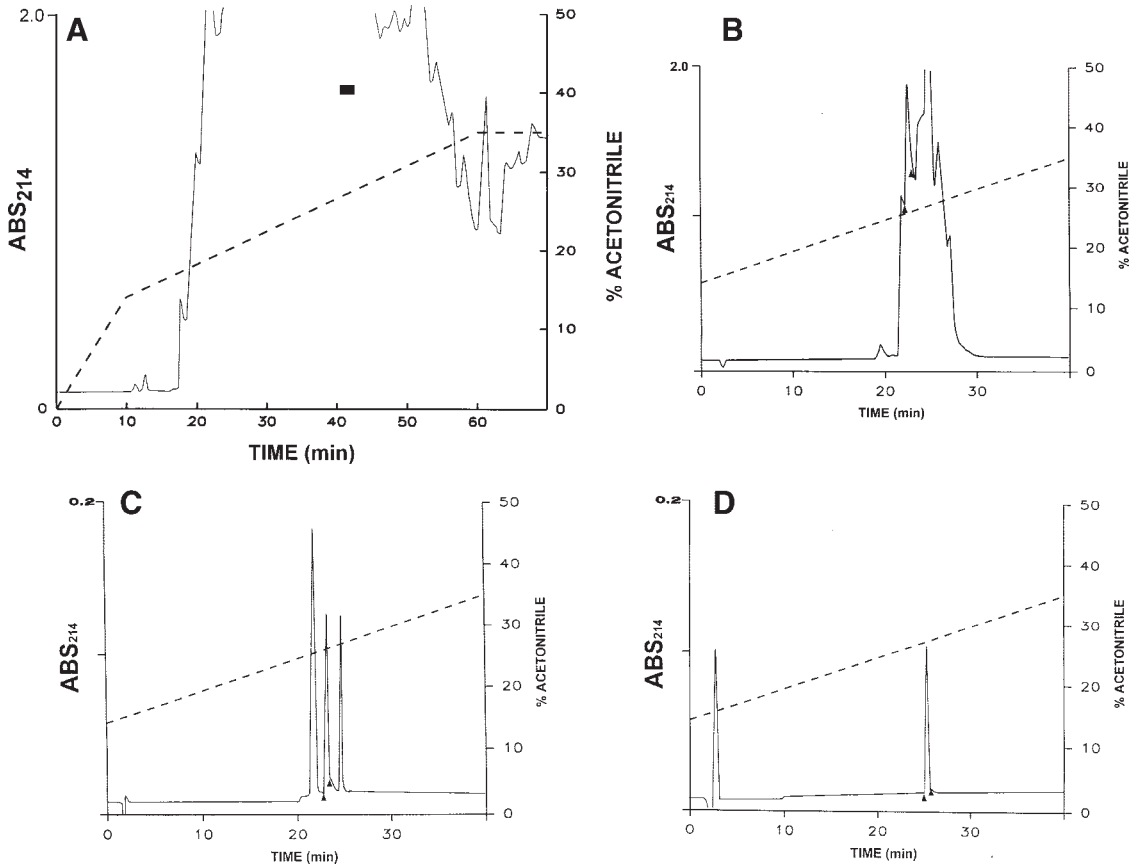

Fig. 1. Purification to near homogeneity of rattlesnake BK by reverse-phase HPLC on Vydac semipreparative C_{18} (*A*), analytical C_4 (*B*), analytical phenyl (*C*), and analytical C_{18} columns (*D*). The bar in *A* shows the fraction with BK-like immunoreactivity and the arrowheads in *B-D* show where peak collection began and ended. The dashed line shows the concentration of acetonitrile in the eluting solvent. ABS_{214}, absorption at 214 nm.

model 491 Procise sequenator (Foster City, CA). Mass spectrometry was performed on a Perkin Elmer Sciex API 150EX single-quadrupole instrument. The accuracy of mass determinations was ±0.02%.

Peptide synthesis. Rattlesnake BK (Val-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg) was synthesized by solid-phase methodology on a 0.025 mmol scale using an Applied Biosystems model 432 synthesizer. Fluorenylmethoxy-carbonyl-labeled 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 by reverse-phase HPLC under the conditions shown in Fig. 1*A*. The peptide was >95% pure, and the correct sequence was confirmed by automated Edman degradation and electrospray mass spectrometry (observed mass 1,016.4 Da, calculated mass 1,016.6 Da).

Hemodynamic Effects of BK in Anesthetized Crotalus

Experimental animals. Studies were undertaken on the South American rattlesnake, *C. durissus terrificus* of both sexes. Snakes were obtained from the Butantan Institute in São Paulo and transported to Universidad Estadual Paulista, Rio Claro, São Paulo (Brazil), where they were housed in a 0.5 × 0.5 m vivariums, and maintained under natural photoperiod at 28 ± 5°C. This study was conducted in accordance with the "APS Guiding Principles for Research Involving Animals and Human Beings." The snakes had free access to water, but food was withheld 1 wk before experimentation. All animals appeared healthy, with body masses ranging from 0.27 to 0.95 kg (0.61 ± 0.06 kg).

Surgery and instrumentation. Snakes were anesthetized by an injection of 30 mg/kg pentobarbital sodium into the tail muscle (Mebumal, Sygehusapotkerne, Denmark). All reflexes disappeared within 20 min, and the animals were then placed in a prone position, so that they could be traecheotomized for artificial ventilation at 4 breaths/min and a tidal volume of 25 ml/kg using a Harvard Apparatus mechanical ventilator. A 5-cm ventral incision was made cranial to the heart, and a PE-50 catheter containing heparinized saline was advanced into the right aortic arch through the vertebral artery. An additional incision was made immediately above the heart. A small branch of the pulmonary artery supplying the dorsal part of the lung was occlusively cannulated with a PE-50 catheter containing heparinized saline. Both 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 of the snake 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, Ithaca, NY) were placed around the left aortic arch (LAo) and the 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 flowmeter (T206). Signals from the pressure transducers and the blood flowmeter were recorded with a Biopac MP100 data-acquisition system (Biopac Systems, Goleta, CA) at 50 Hz.

Calculation of blood flows, stroke volume, and vascular resistances. Because there is only one pulmonary artery in the rattlesnake, measurements of blood flow in the pulmonary artery represent total pulmonary blood flow (Qpul). Total systemic blood flow (Qsys) can be estimated as 3.3 times blood flow in left aortic arch (QLAo) (Galli, Skovgaard, Abe, Taylor, Conlon, and Wang, unpublished observations). Total cardiac output (Qtot) was calculated as Qsys + Qpul. Heart rate (fH) was derived from the instantaneous blood flow trace from the left aortic arch and total stroke volume (Vs,tot; pulmonary + systemic) was calculated as Qtot/fH. When baseline blood flow changes more than baseline blood pressure, which is the case in most in vivo situations, calculated values of conductance provide a better index for comparing vascular tone than resistance (14, 22). Pulmonary and systemic conductance (Gpul and Gsys, respectively) were calculated from mean blood flow and mean blood pressure (Gpul = Qpul/Ppul and Gsys = Qsys/Psys), assuming that central venous blood pressures were negligible.

Experimental protocol. When all hemodynamic parameters had stabilized after surgery, a 0.1 ml/kg injection of 0.9% (wt/vol) saline containing 0.5% (wt/vol) bovine serum was given as a sham injection. Eight snakes then received a series of bolus injections of increasing doses of rattlesnake BK (0.01, 0.03, 0.1, 0.3, 1, 3, and 10 nmol/kg). Hemodynamic variables were allowed to return to baseline levels between each injection.

To investigate the mechanism of action of BK, the effects of treating the snakes with the β-adrenergic receptor antagonist propranolol or the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) were studied. In nine snakes, the following drugs were injected into the arterial catheter in the following order: BK (0.3 nmol/kg), epinephrine (2 μg/kg), propranolol (2 mg/kg), epinephrine (2 μg/kg), and BK (0.3 nmol/kg). The injections of epinephrine were performed to assess the effectiveness of adrenergic blockade, and propranolol was allowed to take effect for 20 min before subsequent injections. In a further eight snakes, the effects of BK (1 nmol/kg) before and after treatment with the nitric oxide synthase inhibitor L-NAME (150 mg/kg) were studied.

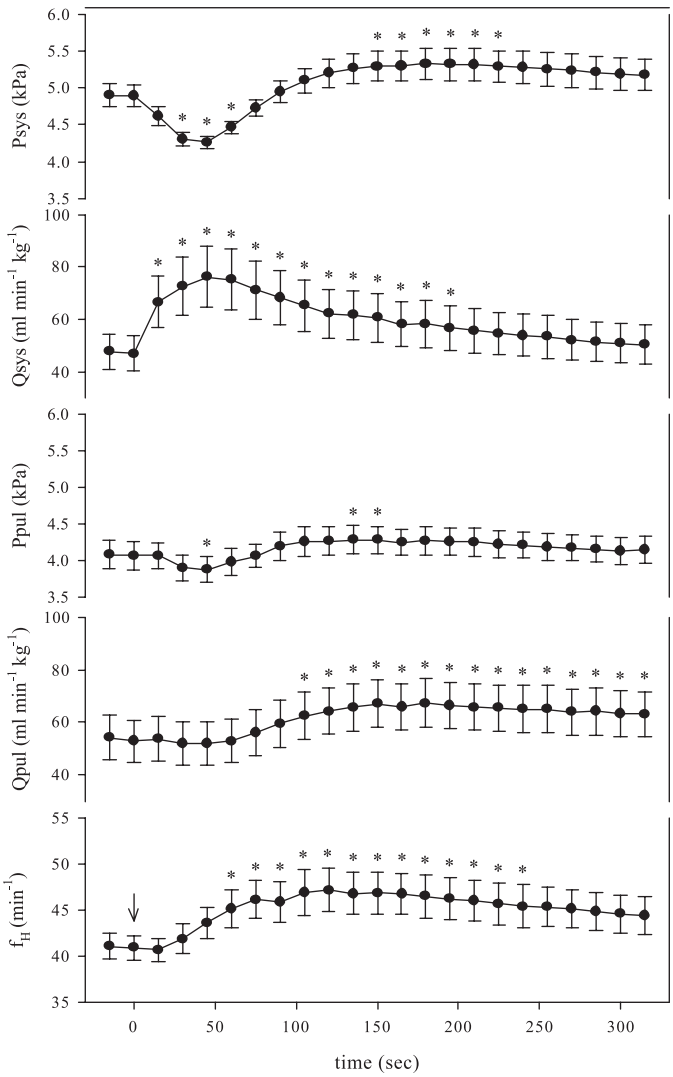

Fig. 2. Mean hemodynamic changes after a bolus injection of 0.3 nmol/kg synthetic rattlesnake BK. Psys, mean pulmonary pressure, Qsys, systemic blood flow, Ppul, mean pulmonary pressure, Qpul, pulmonary blood flow, fH, heart rate. The arrow indicates time of injection. Values are means ± SE; n = 8. *Significant difference of mean from preinjection value.

Data analysis and statistics. All data are presented as means ± SE. Recordings of blood flows and pressures were analyzed using Acq-Knowledge data-analysis software (version 3.23; Biopac, Goleta, CA). Effects on hemodynamic variables were assessed by a one-way ANOVA for repeated measurements followed by a post hoc test to identify values that were significantly different from control values. A limit for significance of $P < 0.05$ was applied.

RESULTS

Characterization and Synthesis of Rattlesnake BK

Purification of rattlesnake BK. The BK-LI in the trypsin-treated rattlesnake plasma, after partial purification on Sep-Pak cartridges, was eluted as a broad, single peak with maximum immunoreactivity at the elution volume of mammalian BK. The immunoreactive fractions were pooled and injected onto a semipreparative Vydac C-18 HPLC column (Fig. 1*A*). BK-LI was eluted in fractions shown by the bar (Fig. 1*A*). These fractions were pooled and chromatographed on an analytical Vydac C_4 column (Fig. 1*B*). The material was heterogeneous, and the BK-LI was associated with the well-defined peak, denoted by arrowheads. Further purification was accomplished on an analytical Vydac phenyl column (Fig. 1*C*), and the BK-LI was associated with the sharp peak, denoted by arrowheads. A final chromatography of rattlesnake BK on analytical Vydac C-18 column (Fig. 1*D*) demonstrated that the peptide had been purified to apparent homogeneity, as indicated by the sharp, symmetrical nature of the peak. The final yield of purified peptide was ~2 nmol.

Structural characterization of rattlesnake BK. The primary structure of rattlesnake BK was established without ambiguity by automated Edman degradation as Val-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg. The amino acid composition of the peptide [found: Gly 1.2 (1), Arg 1.1 (1), Thr 1.2 (1), Pro 2.6 (3), Val 1.0 (1), Phe 1.8 (2) residues/mol peptide] is consistent with the proposed structure. Values in parentheses show the number of residues predicted from the sequence analysis data. The structure was also confirmed by mass spectrometry (observed molecular mass 1,016.5 Da; calculated molecular mass 1,016.6 Da).

Hemodynamic Effects of BK in Anesthetized Crotalus

Cardiovascular effects of rattlesnake BK. Figure 2 shows the recorded hemodynamic changes after a bolus injection of 0.3 nmol/kg synthetic rattlesnake BK. BK produced a rapid (within

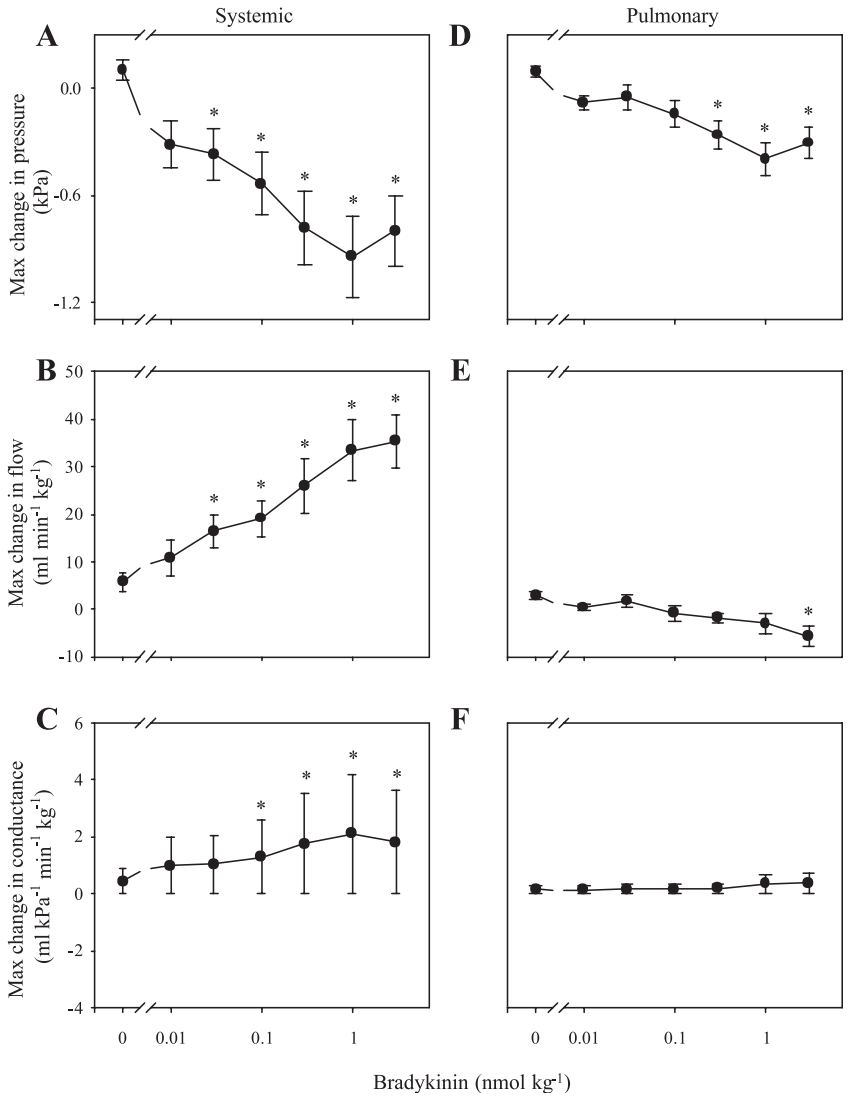

Fig. 3. Hemodynamic effects during the primary phase after a bolus intra-arterial injection of rattlesnake BK on the maximum change in systemic (*A*) and pulmonary (*D*) arterial blood pressure, systemic (*B*) and pulmonary blood flow (*E*), and systemic (*C*) and pulmonary (*F*) vascular resistance as a function of the amount of peptide injected. Values are means ± SE; $n = 8$. *Significant difference of mean from preinjection value.

30 s) and significant fall in Psys that was accompanied by an immediate (within 15 s) increase in Qsys, while *f*H increased gradually at a slower rate. This primary phase of the response lasted for ~120 s and was followed by a further increase in *f*H and a secondary increase in Psys as the vasodilatation subsided and Gsys returned to preinjection values (secondary phase). The two phases were also evident in the pulmonary circulation, but the change in pressures and flows were much smaller than observed in the systemic circulation. The biphasic response depicted in Fig. 2 was present at all dosages of BK, but the magnitude and duration of the changes increased with dose. The mean maximal changes in hemodynamic variables during the primary phase are shown in Figs. 3 and 4, while the mean maximal changes during the secondary response are illustrated in Figs. 5 and 6. There were no effects of administrating the vehicle.

There was a clear dose-dependency of the response during the primary phase, with BK causing a significant increase in Gsys at concentrations at and above 0.1 nmol/kg. This systemic vasodilatation was manifested by a significant increase in Qsys and decrease in Psys (Figs. 3, *A–C*). There were no significant changes in Gpul at any dose tested (Fig. 3*F*). The primary response to BK was also characterized by an increased total stroke volume that was accompanied by significant increases in Qtot and *f*H at higher dosages (Figs. 4, *A–C*). At the highest dose of BK, the ratio of Qpul to Qsys decreased significantly (a right to left shunt) (Fig. 4*D*).

There was also a clear dose-dependency of the responses during the secondary phase (Figs. 5 and 6). A significant rise in Psys occurred at 3 nmol/kg, while Qsys significantly increased at 1 and 3 nmol/kg (Figs. 5, *A–C*). There were no significant changes in the pulmonary circulation (Figs. 5, *D–F*). Doses of BK above 0.1 nmol/kg produced a progressive increase in *f*H, while Vs,tot decreased at the highest dose (3 nmol/kg; Fig. 6, *A* and *B*).

To assess whether the systemic vasodilatation during the primary phase and the tachycardia during the secondary phase were β-adrenergically mediated, we injected the β-adrenergic receptor antagonist propranolol. Injection of propranolol caused significant reductions in both systemic and pulmonary pressures and flows, and heart rate decreased significantly (Table 1). Gsys remained relatively constant after propranolol treatment, while Gpul decreased significantly. Propranolol completely abolished the effect of epinephrine on *f*H, which indicates that the β-adrenergic receptors were fully blocked (Table 1). The effect of epinephrine before and after injection of propranolol is shown in Table 1. In the systemic circulation, epinephrine caused a significant decrease in Gsys, which was of similar magnitude before and after treatment with propranolol (Table 1). In the pulmonary circulation, epinephrine caused a substantial vasodilatation. After treatment with propranolol, however, epinephrine produced a small decrease in Gpul. The rise in Gsys caused by BK (0.3 nmol/kg) was significantly attenuated after treatment with propranolol (Fig. 7). The significant tachycardia observed during the primary and secondary phase of the response was abolished after β-adrenergic blockade.

In further pursuit of the mechanism for the systemic vasodilatory action of BK during the primary phase, we pretreated eight snakes with the nitric oxide synthase inhibitor L-NAME. There was no significant effect of injection of L-NAME on Psys, Qsys, Gsys, Qpul, or Gpul (Table 2); however, there was a significant increase in Ppul and *f*H. BK failed to invoke a significant vasodilatation after injection of L-NAME.

DISCUSSION

The bradykinin-related peptide ([Val^1,Thr^6]BK) generated in plasma from the diamondback rattlesnake *C. atrox* caused a pronounced systemic vasodilatation and tachycar-

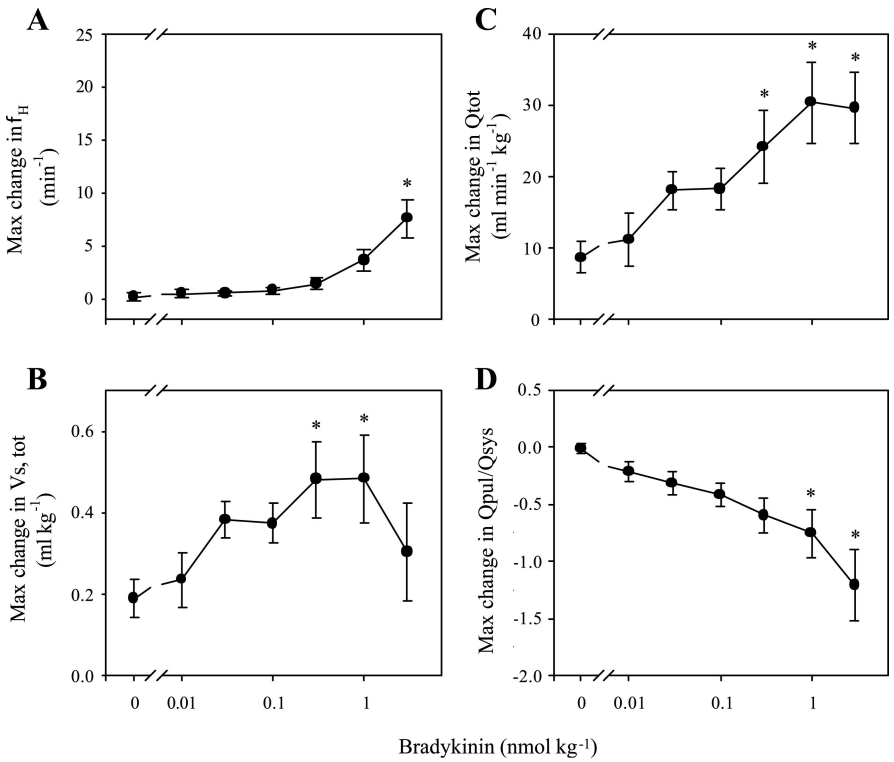

Fig. 4. Hemodynamic effects during the primary phase after a bolus intra-arterial injection of rattlesnake BK on the maximum change in f_H heart rate (*A*); Vs,tot, total stroke volume (*B*); Q_{tot}, total cardiac output (*C*) and Qpul/Qsys, shunt fraction (*D*), as a function of the amount of peptide injected. Values are mean with SE; $n = 8$. *Significant difference of mean from preinjection value.

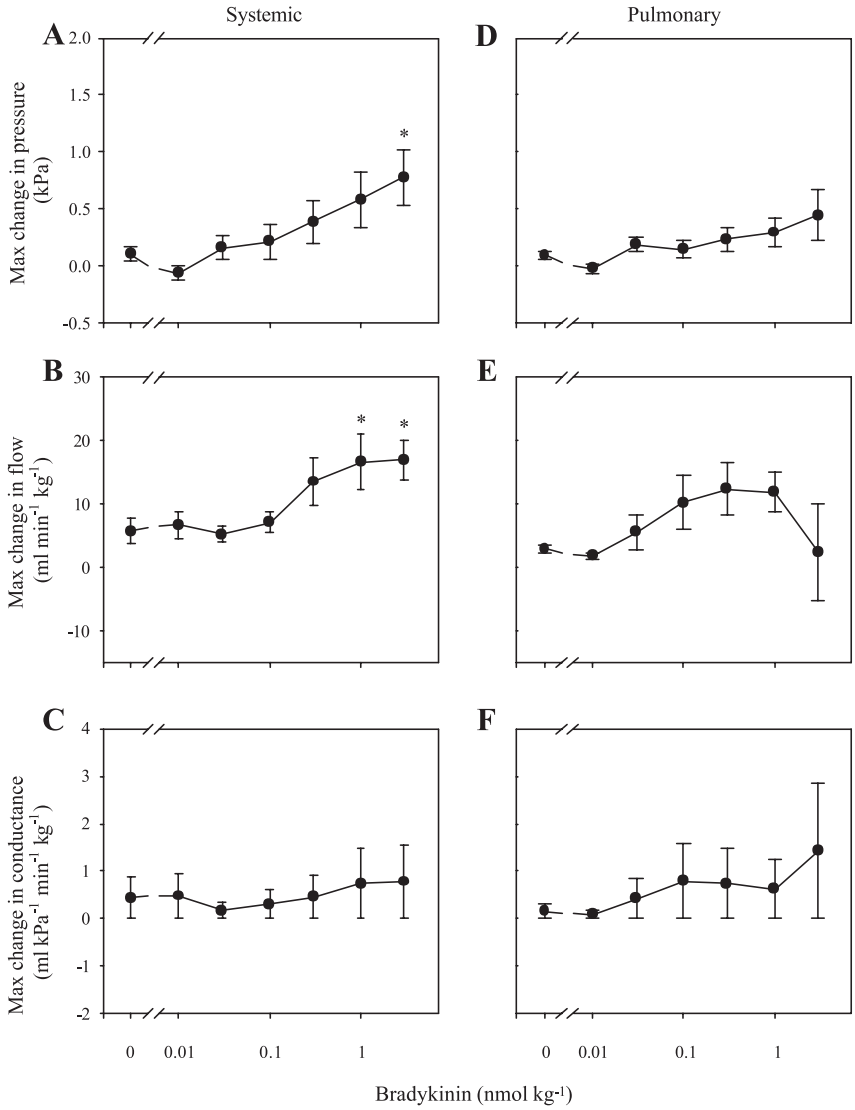

Fig. 5. Maximum change in hemodynamic effects during the secondary phase after a bolus intra-arterial injection of rattlesnake BK as a function of the amount of peptide injected. The meanings of the symbols are the same as in Fig. 3. Values are means ± SE; $n = 8$. *Significant difference of mean from preinjection value.

dia in the South American rattlesnake, *C. durissus terrificus*, but had no effects on the pulmonary circulation. Pretreatment with propranolol greatly attenuated the systemic vasodilatation and abolished the tachycardia, which indicates that a considerable proportion of the cardiovascular responses to BK is caused by adrenergic release and subsequent stimulation of β-adrenergic receptors. In addition, pretreatment with L-NAME attenuated the systemic vasodilatation, indicating that increased NO synthesis is also a contributing factor.

Our study was performed on anesthetized animals. Anesthesia leads to a higher fH and Qpul compared with fully recovered *Crotalus*, which appears to be caused by a withdrawal of vagal tone and elevated sympathetic activity (Wang, Taylor, and Abe, unpublished observation), while autonomic responses, such as barostatic regulation, are virtually abolished. The depressed autonomic function is advantageous for studies on local regulation of the cardiovascular system because there are no reflex responses to altered blood pressure.

Structural Characterization of BK

The bradykinin-related peptide ([Val^1,Thr^6]BK) generated from *Crotalus atrox*, which belongs to the highly evolved family of viperid snakes, was identical to the BK-related peptide from colubrid snakes (15). However, it contained one amino acid substitution (Ala^1 → Val) compared with BK from the reticulated python, which is regarded as being relatively primitive (7). The primary structure of BK also varies among other groups of reptiles (reviewed in 6). Treatment of plasma from the alligator (*A. mississipiensis*) and the turtle (*T. scripta*) with glass beads in the presence of a kininase inhibitor generated [Thr^6]BK (4, 8). In contrast, all attempts to generate BK in the plasma of lizards and snakes through contact with a charged surface have been unsuccessful, suggesting that their blood does not contain a component analogous to mammalian factor XII. However, treatment of heat-denatured plasma from the monitor lizard *Varanus grayi* with trypsin generated [Thr^6]BK, whereas similar treatment of plasma from the Gila monster, *Heloderma suspectum* generated [Leu^2,Thr^6]BK (15).

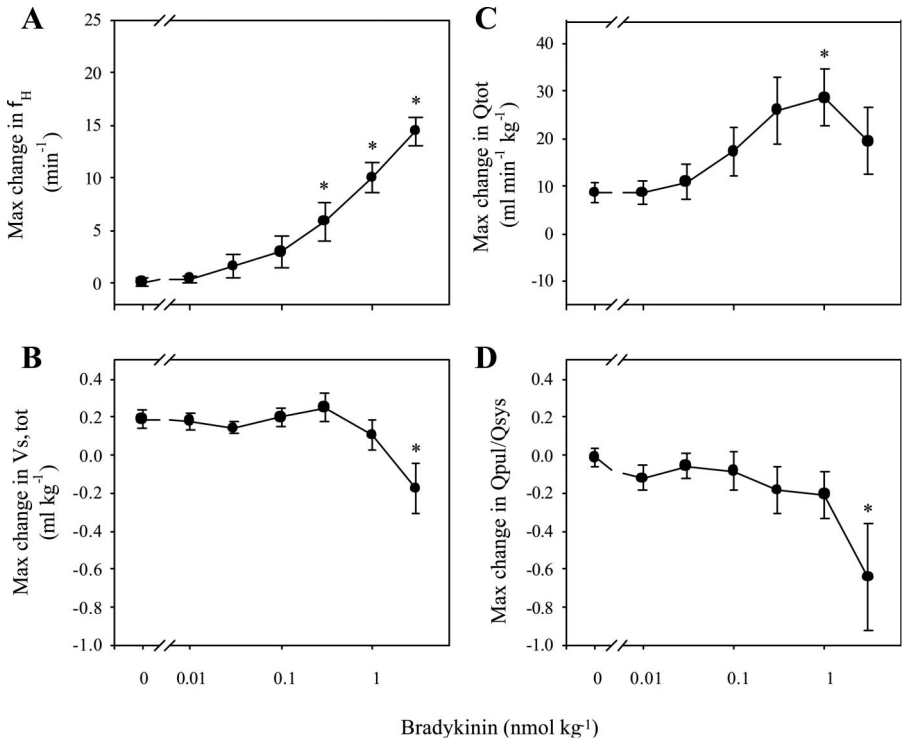

Fig. 6. Maximum change in hemodynamic effects during the primary phase after a bolus intra-arterial injection of rattlesnake BK, as a function of the amount of peptide injected. The meanings of the symbols are the same as in Fig. 3. Values are means ± SE; $n = 8$. *Indicates significant difference of mean from preinjection value.

Cardiovascular Responses to BK

Infusion of BK into rattlesnakes caused an initial vasodilatation of the systemic circulation that was manifested as a fall in systemic arterial pressure and a rise in Qsys. This primary response was followed by a subsequent rise in Psys as *f*H increased and Gsys returned to baseline values. These responses closely resemble the biphasic response of mammals but differ somewhat from other species of reptiles. Turtles and pythons also exhibit a systemic vasodilatation when injected with their native BK, but the changes in blood pressure and heart rate differ. Turtles had a monophasic response, where the vasodilatation is associated with a fall in Qsys, while Psys remained constant (8). There was, however, no tachycardia associated with BK injection in turtles. In pythons, BK also caused a pronounced vasodilatation, but Psys increased as a result of a profound tachycardia and a rise in Qsys (28).

The BK-induced systemic vasodilatation in the rattlesnake was significantly attenuated by prior treatment with propranolol (Fig. 7), indicating that a considerable portion of this response is mediated by stimulation of β-adrenergic receptors. This would indicate that BK causes release of catecholamines from adrenergic presynaptic neurons or the adrenal medulla. Not having measured plasma concentrations of epinephrine and norepinephrine in response to BK, we cannot discern between these two possibilities. However, in mammals and pythons, injection of BK leads to a rise in circulating catecholamines, which is released from sympathetic neurons (27, 28).

Given that part of the BK-induced vasodilatation persisted after treatment with propranolol, other mechanisms seem to be involved in this response. BK failed to induce a significant vasodilatation after injection of L-NAME, implying that increased synthesis of NO contributes to the cardiovascular response to BK. The mechanism by which BK dilates the systemic vasculature differs among the different groups of vertebrates. As in the rattlesnake, the systemic vasodilatation caused by BK in the python (*P. regius*) was abolished after treatment with the β-adrenergic receptor antagonist sotalol, indicating that this response is mediated by stimulation of β-adrenergic receptors. However, in mammals, the vasodilator effects of BK have been attributed to both the activation of prostaglandins and through NO release (11, 20).

Having injected epinephrine and propranolol to unravel the mechanisms behind the action of BK, our study also yields

Table 1. *Hemodynamic changes following injection of epinephrine, propranolol, and epinephrine with propranolol*

	Ppul, kPa	Psys, kPa	Qpul, ml·kg^{-1}·min^{-1}	Qsys, ml·kg^{-1}·min^{-1}	Gpul, ml·kPa^{-1}·min^{-1}·kg^{-1}	Gsys, ml·kPa^{-1}·min^{-1}·kg^{-1}	*f*H, beats/min
Preinjection	2.48±0.29	3.50±0.45	18.7±4.2	24.2±4.2	6.9±1.0	9.2±2.7	8.5±1.4
Epinephrine	3.72±0.16*	5.69±0.56*	51.5±10.2*	31.6±5.4*	13.4±2.4*	6.4±1.7*	53.2±1.1*
Preinjection	2.91±0.22	4.53±0.43	33.5±7.9	29.0±4.4	10.8±2.2	7.5±2.0	46.7±1.2
Propranolol	2.59±0.21*	3.11±0.31*	21.9±6.1*	17.3±3.0*	7.6±1.7*	6.0±1.1	29.7±2.2*
Preinjection	2.60±0.21	3.04±0.27	21.9±6.1	16.6±3.0	7.7±1.7	5.8±1.0	28.8±2.2
Epinephrine	4.07±0.17*	4.71±0.39*	31.6±10.2	14.0±3.0	7.4±2.1	2.9±0.5*	29.7±2.2

Values are means ± SE; $n = 9$. Ppul, mean pulmonary blood pressure; Psys, mean systemic blood pressure; Qpul, pulmonary blood flow; Qsys, systemic blood flow; Gpul, pulmonary conductance; Gsys, systemic conductance; and *f*H, heart rate. *Significantly different ($P < 0.05$) from preinjection value.

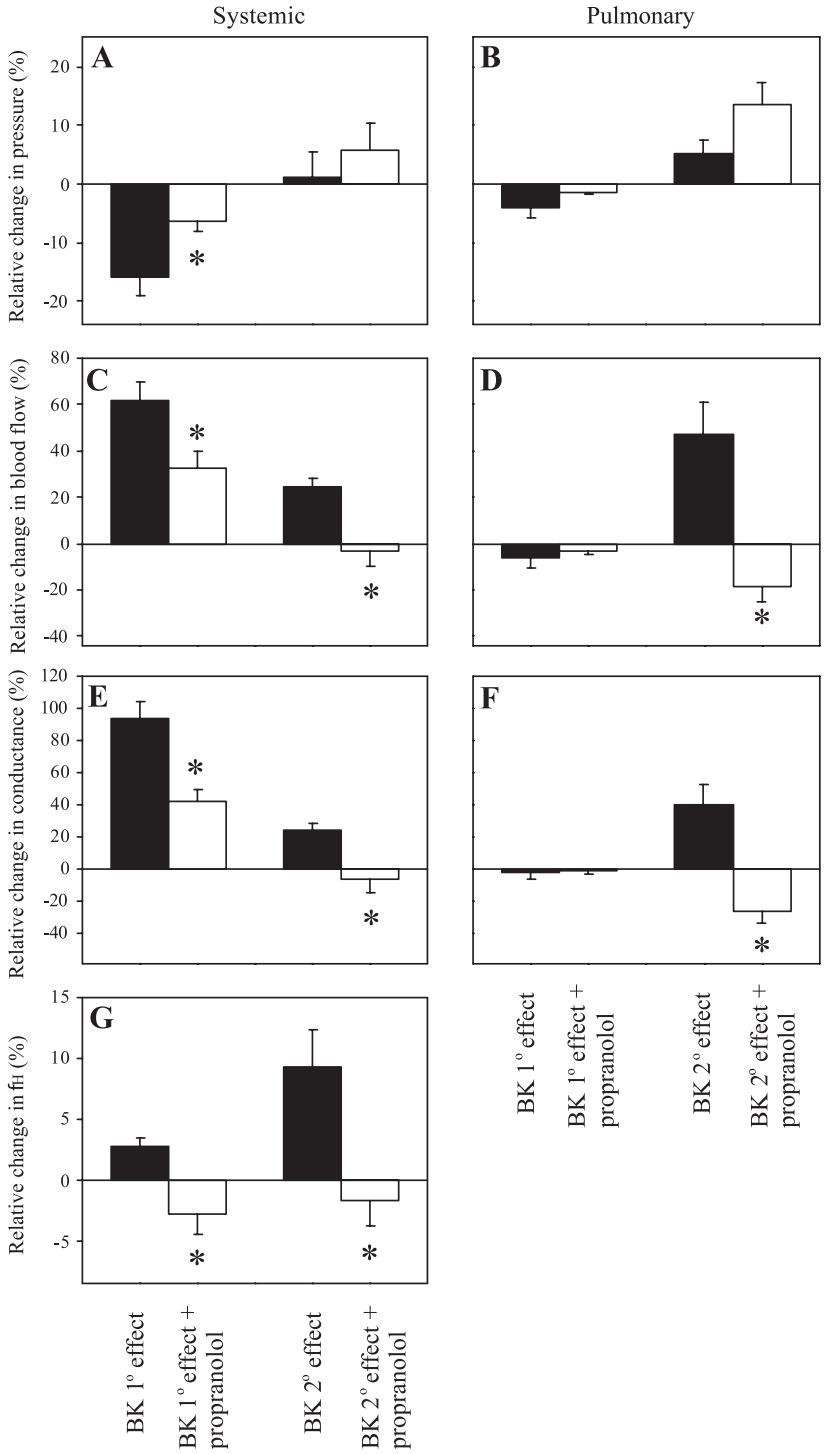

Fig. 7. Hemodynamic changes during the primary (1°) and secondary (2°) phase after a bolus injection of rattlesnake BK (0.3 nmol/kg) with and without treatment with propranolol (2 mg/kg). Relative change is shown in systemic (*A*) and pulmonary (*B*) arterial blood pressure; systemic (*C*) and pulmonary (*D*) blood flow; systemic (*E*) and pulmonary (*F*) vascular resistance; *f*H, heart rate (*G*). Solid bars indicate preinjection values, and open bars indicate values after injection of BK. Values are means ± SE; $n = 8$. *Significant difference of mean from preinjection value.

Table 2. *Hemodynamic changes following injection of BK, L-NAME, and BK with L-NAME*

	Ppul, kPa	Psys, kPa	Qpul, ml·kg^{-1}·min^{-1}	Qsys, ml·kg^{-1}·min^{-1}	Gpul, ml·kPa^{-1}·min^{-1}·kg^{-1}	Gsys, ml·kPa^{-1}·min^{-1}·kg^{-1}	*f*H, beats/min
Preinjection	3.1±0.1	4.1±0.7	56.3±13.5	95.2±19.0	19.6±5.9	23.5±5.2	43.4±4.2
BK (0.1 nmol/kg)	3.0±0.2	3.6±0.2*	57.4±14.3	127.4±19.7*	20.2±6.1	35.6±5.5*	48.1±4.5*
Preinjection	3.2±0.2	4.2±0.2	63.9±14.3	78.7±11.2	21.0±5.1	19.3±3.0	44.2±3.7
L-NAME	3.4±0.3*	4.6±0.4	70.4±17.4	77.8±16.0	22.1±5.5	17.3±3.8	47.9±3.7*
Preinjection	3.2±0.3	4.3±0.3	62.8±20.4	77.8±12.2	21.6±7.6	19.0±3.4	43.5±3.5
BK (0.1 nmol/kg)	3.1±0.2	4.0±0.3	60.9±20.4	100.6±12.2	21.3±8.1	27.2±4.8	45.6±3.4

Values are means ± SE; $n = 8$. *Significantly different ($P < 0.05$) from preinjection value. BK, bradykinin; L-NAME, N^G-nitro-L-arginine methyl ester.

some information regarding adrenergic tone in rattlesnakes. Injection of epinephrine caused a significant systemic vasoconstriction that persisted after treatment with propranolol, indicating that this response is mediated by stimulation of α-adrenergic receptors. Furthermore, injection of propranolol caused a decrease in Gsys, indicating a basal β-adrenergic tone on the systemic vasculature. Similar responses have been reported in other reptiles (13, 16, 21, 23, 28).

The tachycardia caused by BK (Fig. 7) was abolished by propranolol in rattlesnakes, indicating that it is caused by stimulation of β-adrenergic receptors. This is also the case in python, where the tachycardia was virtually abolished after treatment with sotalol (28) and where circulating catecholamines increased in response to BK (28). Similarly, the tachycardia associated with the secondary response to BK in mammals has been ascribed to presynaptic release of catecholamines (27).

This is the first study to address the role of BK in the pulmonary circulation in a nonmammalian vertebrate. The pulmonary circulation of rattlesnakes was relatively unaffected by BK. In conjunction with this, several other vasoactive substances appear to have small effects on the pulmonary circulation in reptiles. In pythons, injection of neuropeptide γ causes a dose-dependent vasodilatation in the systemic circulation, while it has little effect on the pulmonary vasculature (Skovgaard, Galli, Taylor, Conlon, and Wang, unpublished observations). Similarly, in the American alligator, injection of endothelin-3 caused a decrease in systemic resistance, while the pulmonary circulation was unaffected (24). In *Crotalus*, NO contributes to the resting tone of the systemic circulation, and infusion of the NO donor sodium nitroprusside caused a systemic vasodilatation, but there were no effects on the pulmonary circulation (Galli, Skovgaard, Abe, Taylor, Conlon, and Wang, unpublished observations). Finally, in most reptiles, sympathetic regulation seems to be more pronounced in the systemic circulation compared with the pulmonary circulation. In the turtle, *T. scripta*, injection of catecholamines generally causes a small vasodilatation in the lungs, whereas the systemic effects are marked (1, 3, 5, 18, 23). Isolated pulmonary arteries from the snake, *Elaphe obsolete*, and the lizard, *Trachydosaurus rugosus*, exhibit a β-adrenergically mediated pulmonary relaxation, while adrenergic stimulation failed to invoke a response in the pulmonary artery of the tortoise, *Chelodina longicollis* (1). However, in the rattlesnake, epinephrine caused a marked vasodilation in the pulmonary circulation that was abolished with propranolol (Table 2).

Perspectives

In both mammals and those species of reptiles studied, BK consistently causes a primary dose-dependent systemic vasodilatation. The sensitivity of the cardiovascular system of the python and the rattlesnake to BK [threshold 30 and 100 nmol/kg, respectively (28)] implies that this peptide may have a physiologically relevant role for cardiovascular regulation. In mammals, circulating levels of BK rise in response to tissue injury, stimulating the release of prostaglandins and the synthesis of NO, which alter distribution of blood flow to the damaged tissue area (11, 20, 25). In snakes, BK seems to exert its vasodilatory effects through stimulation of adrenergic release from sympathetic neurons. Nevertheless, the resulting cardiovascular effects are similar. Thus we would speculate that although the mechanisms through which BK acts may differ somewhat between mammals and snakes, the resulting cardiovascular actions and its functional significance in the response to tissue injury may be the same.

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REFERENCES

1. **Berger PJ.** Autonomic innervation of the visceral and vascular smooth muscle of the lizard lung. *Comp Gen Pharmacol* 4: 1–10, 1973.
2. **Bhoola KD, Figueroa CD, and Worthy K.** Bioregulation of kinins: kallikreins, kininogens and kininases. *Pharmacol Rev* 44: 1–80, 1992.
3. **Burggren W.** The pulmonary circulation of the chelonian reptile: morphology, haemodynamics and pharmacology. *J Comp Physiol [A]* 116: 303–323, 1977.
4. **Comeau S, Lance VA, Hicks JW, and Conlon JM.** Purification and biological activity of alligator bradykinin. *Am J Physiol Regul Integr Comp Physiol* 263: R400–R404, 1992.
5. **Comeau SG and Hicks JW.** Regulation of central vascular blood flow in the turtle. *Am J Physiol Regul Integr Comp Physiol* 267: R569–R578, 1994.
6. **Conlon JM.** Bradykinin and its receptors in non-mammalian vertebrates. *Regul Pept* 79: 71–81, 1999.
7. **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.
8. **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.
9. **Crossley D, Wang T, and Altimiras J.** Role of nitric oxide in the systemic and pulmonary circulation of anaesthetised turtles (*Trachemys scripta*). *J Exp Zool* 286: 683–689, 2000.
10. **Diniz CR and Carvalho IF.** A micromethod for determination of bradykininogen under several conditions. *Ann NY Acad Sci* 104: 77–89, 1963.
11. **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.
13. **Hicks JW and Farrell AP.** The cardiovascular responses of the red-eared slider (*Trachemys scripta*) acclimated to either 22 or 5°C. II. Effects of anoxia on adrenergic and cholinergic control. *J Exp Biol* 203: 3775–3784, 2000.
14. **Lautt WW.** Resistance or conductance for expression of arterial vascular tone. *Microvasc Res* 37: 230–236, 1989.
15. **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.
16. **Lillywhite HB and Donald JA.** Neural regulation of arterial blood pressure in snakes. *Physiol Zool* 67: 1260–1283, 1994.
17. **Marceau F, Bachvarov DR.** Kinin receptors. *Clin Rev Allergy Immunol* 16: 385–401, 1998.
18. **Milsom WK, Langille BL, and Jones DR.** Vagal control of pulmonary vascular resistance in the turtle *Chrysemys scripta*. *Can J Zool* 55: 359–367, 1977.
19. **Murphy R, Fu WJ, Lathrop A, Feltham JV, and Kovac V.** Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial DNA genes. In *Biology of the Vipers,* edited by Schuett GW, Höggren M, Douglas ME, and Greene HW. Eagle Mountain, UT: Eagle Mountain Publishing, 2002, pp. 69–92.
20. **Needleman P, Key SL, Denny SE, Isakson PC, and Marshall GR.** The mechanism and modification of bradykinin-induced coronary vasodilation. *Proc Natl Acad Sci USA* 72: 2060–2063, 1975.

21. **Nilsson S.** *Autonomic Nerve Function in the Vertebrates.* New York: Springer Verlag, 1983.
22. **O'Leary DS.** Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol Heart Circ Physiol* 260: H632–H637, 1991.
23. **Overgaard J, Stecyk JA, W, Farrell AP, and Wang T.** Adrenergic control of the cardiovascular system in the turtle *Trachemys scripta*. *J Exp Biol* 205: 3335–3345, 2002.
24. **Platzack B, Wang Y, Crossley D, Lance V, Hicks JW, and Conlon JM.** Purification, characterization and cardiovascular actions of endothelin-1 and endothelin-3 from the American alligator. *Am J Physiol Regul Integr Comp Physiol* 282: R594–R602, 2002.
25. **Sharma JN, Sharma J.** Cardiovascular properties of the kallikrein-kinin system. *Curr Med Res Opin* 18: 10–17, 2002.
27. **Uchida Y and Murao S.** Bradykinin-induced excitation of afferent cardiac sympathetic fibers. *Jap Heart J* 15: 84–91, 1974.
28. **Wang T, Axelsson M, Jensen J, and Conlon JM.** Cardiovascular actions of python bradykinin and substance P in the anesthetized python, *Python regius*. *Am J Physiol Regul Integr Comp Physiol* 279: R531–R538, 2000.