The Journal of Experimental Biology 201, 2235–2242 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1547

RELATIONSHIPS BETWEEN BLOOD PRESSURE AND HEART RATE IN THE SALTWATER CROCODILE *CROCODYLUS POROSUS*

JORDI ALTIMIRAS[1,*], CRAIG E. FRANKLIN[2] AND MICHAEL AXELSSON[3]

[1]*Department of Zoophysiology, University of Aarhus, Århus C, Denmark*, [2]*Department of Zoology, University of Queensland, Brisbane, Australia* and [3]*Department of Zoophysiology, University of Göteborg, Göteborg, Sweden*

*e-mail: Jordi.Altimiras@biology.aau.dk

Accepted 26 May; published on WWW 14 July 1998

Summary

The cardiac limb of the baroreflex loop was studied in the saltwater crocodile *Crocodylus porosus*. The classical pharmacological methodology using phenylephrine and sodium nitroprusside was used to trigger blood pressure changes, and the resulting alterations in heart rate were analysed quantitatively using a logistic function. Interindividual differences in resting heart rates and blood pressures were observed, but all seven animals displayed clear baroreflex responses. Atropine and sotalol greatly attenuated the response. A maximal baroreflex gain of 7.2 beats min^{-1} kPa^{-1} was found at a mean aortic pressure of 6.1 kPa, indicating the active role of the baroreflex in a wide pressure range encompassing hypotensive and hypertensive states. At the lowest mean aortic pressures (5.0 kPa), the synergistic role of the pulmonary-to-systemic shunt in buffering the blood pressure drop also contributes to blood pressure regulation.

Pulse pressure showed a better correlation with heart rate and also a higher gain than mean aortic, systolic or diastolic pressures, and this is taken as an indicator of the existence of a differential control element working simultaneously with a linear proportional element.

Key words: crocodilian, reptile, baroreflex, heart rate, blood pressure, shunt, nitroprusside, phenylephrine, *Crocodylus porosus*.

Introduction

In vertebrates, short-term imbalances in blood pressure are regulated by reflex changes in cardiac output and peripheral resistance, the so-called cardiac limb and peripheral limb of the baroreflex, respectively. Van Vliet and West (1994) reason that without this feedback controller the cardiovascular system would be vulnerable to acute hypertension that could result in vascular lesions, increased capillary pressures and oedema and impaired flow autoregulation among other problems.

Baroreflex responses have been identified in many vertebrate species (see review by Bagshaw, 1985). In reptiles, the anatomy of baroreceptive fibres as well as their functional responses have been studied in turtles, lizards and snakes (Stephens *et al.* 1983; Millard and Moalli, 1980; Berger *et al.* 1980; Backhouse *et al.* 1989). Little is known in crocodilians, although the existence of a functional baroreflex response is hardly disputed.

In mammals, and probably birds, the existence of myelinated baroreceptors (with thresholds well below normal arterial pressures) permits compensation for acute hypotension. This is believed to protect the tissues from ischaemia, which could result from a drop in the driving pressure and the high metabolic rates of endothermic species (Van Vliet and West, 1994).

The involvement of the baroreflex in counteracting hypotension might also be critical in crocodilians. Although crocodilians have a completely divided ventricle, pulmonary bypass of less-oxygenated blood (pulmonary-to-systemic shunt) is still possible through the left aorta, and this appears to occur to some extent in resting animals (White, 1969; Jones and Shelton, 1993) and is possibly even more pronounced during diving. As the magnitude of the pulmonary-to-systemic shunt is determined by a balance between the systemic and pulmonary resistance (Shelton and Jones, 1991; Axelsson and Franklin, 1997), hypotension would result in an increase in the shunt flow and systemic hypoxaemia. We reasoned that the baroreflex could be important to counteract such changes and to prevent systemic ischaemia.

The present study was designed with two main goals (1) to evaluate the baroreflex quantitatively, estimating the gain of the cardiac limb of the reflex at different mean systemic pressures; and (2) to determine whether the baroreflex is capable of responding to hypotensive episodes and of preventing systemic ischaemia.

Materials and methods

Experimental animals

Crocodylus porosus Schneider (body mass 2015±121 g; mean ± S.E.M., N=7) were obtained from the Cairns Crocodile

Farm, Cairns, Australia. The crocodiles were transported to the University of Queensland, where they were housed outdoors in a large (4 m diameter) fibreglass tank. They were kept in fresh water heated to 28 °C and had access to basking platforms. The animals were fed once a week.

Surgical protocol

Crocodiles were intubated with a 5 mm rubber tube after local application of lignocaine (20 mg ml^{-1}, Lignomav) on the glottis. They were anaesthetised using 5 % halothane in oxygen, until the eye reflexes and muscular tone were dampened, and intermittently ventilated with oxygen:halothane (1 %) thereafter.

To obtain the electrocardiographic trace (ECG), two resin-coated stainless-steel wires (80 cm long, 0.27 mm diameter, purchased from Driver Harris S.A., insulated by Aismalibar S.A.) were inserted subdermally on the ventral side in a caudocephalic direction using an 18 gauge needle. Insulation at the tip of each electrode was stripped away over 3 cm to make a good contact surface to pick up the electrocardiogram (ECG) signal.

To obtain an impedance pneumographic trace (IP), two stainless-steel wires, with 1 cm of insulation removed from the tips, were inserted subdermally on the flank of the animal, midway between the front limb and the hind limb.

All the wires were looped and sutured onto the skin at the base of the implantation site and also on the dorsal surface, where they were passed through a short length of PE 60 polyethylene cannula. Small connectors (2 mm diameter) were soldered at the end of each wire.

To catheterise the femoral artery and vein, a 4 cm incision was made on the posterior part of the right hindlimb. Both vessels lie between the iliotibialis and the femorotibialis muscles (Guibé, 1970). The femoral artery was freed of connective tissue and occlusively cannulated in an upstream direction with a polyurethane cannula (1.22 mm outer diameter, tipped with a 0.8 mm polyurethane cannula). The artery was rinsed with heparinised saline (100 i.u. ml^{-1} heparin in 0.9 % NaCl solution). The same protocol was followed to cannulate the femoral vein, which was occlusively cannulated in the downstream direction.

Both cannulae were externalised through a pair of holes on the skin at the base of the right hindlimb and connected to two titanium ports that were, in turn, secured to dorsal scutes with 2-0 silk sutures. Finally, the incision was closed with single stitches, the area was cleaned with 70 % ethanol and powder antibiotic was topically applied (Cicatrin, Wellcome). The double cannulation had no visible effect on the movement of the limb.

The whole surgical protocol took approximately 30–40 min and was carried out under sterile conditions. The research protocol was approved by the University of Queensland's Animal Ethics and Experimentation Committee, Permit no. ZOO/688/95/ARC.

Animal maintenance

After recovery from anaesthesia, the animals were individually housed in rectangular plastic tanks, where they were kept for a minimum of 36 h without disturbance. During the post-operative and recording periods, 0.2 ml of amoxycillin was given intramuscularly every other day (150 mg ml^{-1} Amoxil).

For the first 24 h after surgery, the animals were kept in dry tanks to prevent water from seeping into the wounds and causing infections. Fresh water was added subsequently, covering the limbs but not the back of the animal. The crocodiles were unable to dive during the experiment.

Signal recording

At the start of the experimental protocol, the ECG and IP leads were connected to a Grass EEG amplifier unit and an impedance converter (model 2991, UFI) respectively. The arterial catheter was connected to a Statham pressure transducer calibrated against a static water column. The three signals (ECG, IP and blood pressure, Pb) plus heart rate (f_H), which was derived from the pulsatile pressure signal using a tachograph channel, were displayed on a four-channel Grass chart recorder and simultaneously stored on a computer (Toshiba T6400DX) at 250 Hz using a 12-bit data-acquisition card (LabPC+) in combination with LabView v.3.1.1 (National Instruments, Austin, TX, USA).

Pharmacological protocol to test baroreflex gain

After a 10 min recording period (which we will refer to as 'control'), 1 ml of 0.9 % NaCl was injected into the femoral vein through the titanium port to check whether the infusion volume in itself induced cardiovascular changes. Serial increasing doses of phenylephrine (hydrochloride salt, Sigma; 5, 10, 20, 30 and 60 μg kg^{-1}) and sodium nitroprusside (Sigma; 5, 10, 25 and 50 μg kg^{-1}) were then injected. Pb, ECG and IP were recorded for 10 min after each infusion (0.2 ml bolus followed by 0.8 ml of 0.9 % NaCl solution). The next dose was not injected until the recorded variables had returned to pre-injection values, which usually took less than 10 min for all doses except the largest ones. Special care was taken to avoid the interference of ventilation and the large shifts in f_H associated with it (Huggins *et al.* 1970). In case the animal ventilated during the first 2 min after injection, the trial was repeated a second or a third time if necessary.

In two of the animals, the same protocol was repeated with the largest dose of each drug (60 μg kg^{-1} phenylephrine and 50 μg kg^{-1} sodium nitroprusside) after full autonomic blockade of the heart (3 mg kg^{-1} sotalol hydrochloride, Bristol Myers, and 1.2 mg kg^{-1} atropine sulphate, Sigma).

Calculation of baroreflex gain

Heart rate (f_H), diastolic pressure (P_D), systolic pressure (P_S) and pulse pressure (P_P) were obtained before (control value) and after each pharmacological challenge (treatment value). The maximal effect was always obtained within 1 min after infusion.

Baroreflex gain was estimated by fitting all the data to a four-variable sigmoidal logistic function (Reid, 1996):

$$f_H = \frac{A-D}{1+(\overline{P}_A/C)^B} + D \quad (1)$$

where A and D are the maximal and minimal f_H, respectively,

attained by the baroreflex, B is the maximum slope of the linear part of the function, C is $\overline{P_A}$ at the midpoint in the f_H range and $\overline{P_A}$ is the mean aortic pressure calculated as:

$$\overline{P_A} = \tfrac{2}{3}P_D + \tfrac{1}{3}P_S\,. \quad (2)$$

The best fit was computed using a Quasi-Newtonian iterative method in the non-linear estimation module of Statistica v.5.0 (StatSoft Inc.). The estimation error was minimised using the least-squares criterion, and the goodness of the fit is reported as the coefficient of determination (r^2, the ratio of the regression sum of squares to the total sum of squares).

The gain (G_{50}) of the baroreflex at P_{b50} (i.e. when $\overline{P_A}=C$) was calculated as:

$$G_{50} = \frac{-B(A-D)}{4C} \quad (3)$$

and normalised by recalculating gain as the percentage change in heart rate per unit change in mean arterial pressure (Berger *et al.* 1980). The formula given by Smith *et al.* (1981) to calculate normalised gain G (% kPa^{-1}) can be easily adapted to the four-variable sigmoidal model:

$$G = 100B/D\,. \quad (4)$$

Results

The resting cardiovascular variables of the seven animals used in the study are reported in Table 1. The values correspond to the mean values over a 30 min period in which the animals were not disturbed, and only periods when the animal was not breathing were accounted for. Clear inter-individual differences in blood pressure and heart rate were noted during the study, but could not be explained by differences in any of the controlled variables. $\overline{P_A}$ ranged from 5.8 to 8.4 kPa, while f_H fluctuated between 14.7 and 25.8 beats min^{-1}.

The injection of 1 ml of saline (results not shown) had no observable effects. Using an estimated total blood volume of 7.3 % (Altman and Dittmer, 1971) in a standard 2 kg animal, the volume infused was of the order of 1 % of the total blood volume.

Table 1. *Control variables of the seven crocodiles*

Animal	f_H	P_D	P_S	$\overline{P_A}$	P_P
1	19.0±4.9	6.5±0.4	9.1±0.8	7.4±0.6	2.5±0.4
2	17.1±1.6	7.3±0.5	10.4±0.9	8.4±0.6	3.1±0.5
3	25.8±2.5	6.5±0.5	9.2±0.6	7.4±0.5	2.6±0.3
4	14.7±4.3	6.2±0.6	8.8±1.0	7.0±0.8	2.6±0.5
5	20.1±3.1	5.8±0.6	8.9±0.8	6.8±0.6	3.1±0.5
6	19.6±2.3	4.8±0.4	7.7±0.4	5.8±0.4	2.9±0.5
7	17.6±7.6	6.1±0.9	9.1±1.3	7.1±1.1	3.0±0.5
Mean	19.1±3.5	6.2±0.8	9.0±0.8	7.1±0.8	2.8±0.2

Values are means ± S.D., N=7.

f_H, heart rate; P_D, diastolic pressure; P_S, systolic pressure; $\overline{P_A}$, mean aortic pressure; P_P, pulse pressure.

Pharmacological alterations in blood pressure triggered clear baroreflex responses. The alpha-adrenoceptor agonist phenylephrine increased $\overline{P_A}$ by inducing a generalised vasoconstriction of the peripheral vasculature, while sodium nitroprusside induced a general vasodilation in response to the release of nitric oxide (see two example traces in Fig. 1).

All the animals responded to the pressure challenges with reflex reciprocal changes in heart rate. This is shown in Fig. 2A, where each line represents the $\overline{P_A}/f_H$ relationships in control conditions and after injection of phenylephrine and sodium nitroprusside for each of the seven animals.

The range of pressures and heart rates measured in each individual precluded the calculation of individual baroreflex sensitivity. As an alternative, we pooled the data from the seven animals and constructed a pooled baroreflex curve using the four-variable sigmoidal logistic function (equation 1) (Fig. 2B). The maximal gain of the baroreflex was 7.2 beats min^{-1} kPa^{-1}, and this was found at a $\overline{P_A}$ of 6.1 kPa (Table 2), slightly below the control $\overline{P_A}$ of 7.1 kPa. The same logistic function was used to analyse the relationship between phenylephrine, P_S and P_P and f_H.

The goodness of the fit between the experimental data and the model is determined by the coefficient of determination r^2 (Sokal and Rohlf, 1995), which ranged between 0.403 (P_D/f_H) and 0.513 (P_P/f_H). This indicates that 40–50 % of the variations in f_H are explained by variations in blood pressure.

The baroreflex was blunted by pharmacological autonomic blockade with atropine and sotalol, as shown in Fig. 3 for the two animals tested. Baroreflex sensitivity after blockade was 0.9 beats min^{-1} kPa^{-1} compared with the intact baroreflex gain of 7.2 beats min^{-1} kPa^{-1}.

Aside from the sensitivity of the baroreflex, it is important

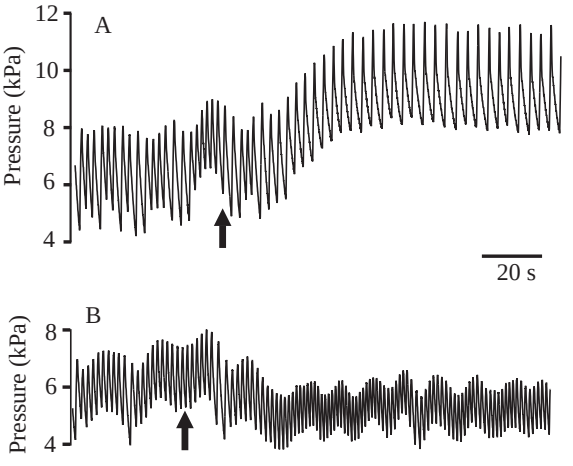

Fig. 1. Original traces from animal 7 (A) after injection of 30 µg kg^{-1} phenylephrine and (B) after injection of 50 µg kg^{-1} sodium nitroprusside. The arrows mark the time of injection. Note the decrease in heart rate after blood pressure was raised in response to phenylephrine and the increase after blood pressure was decreased in response to sodium nitroprusside.

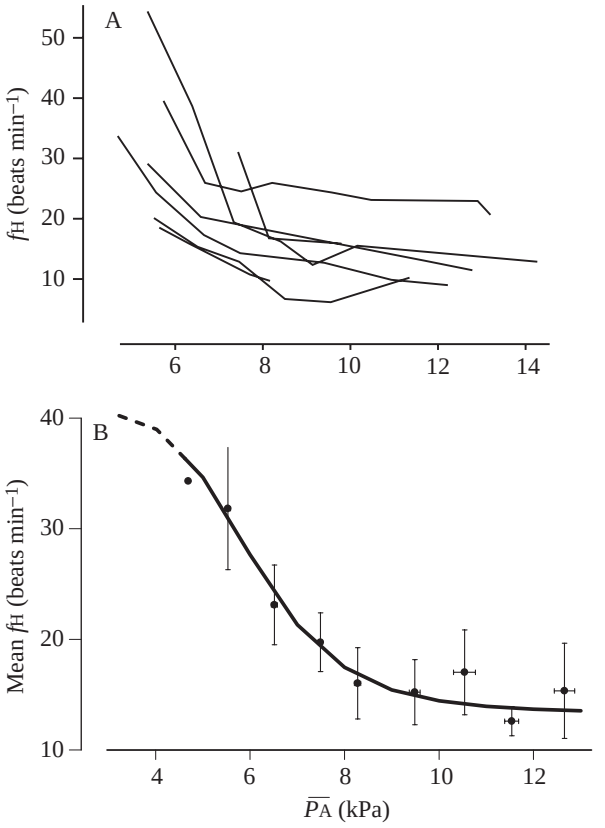

Fig. 2. (A) Individual responses of each of the seven animals after pharmacologically induced changes in blood pressure. (B) Relationship between mean arterial blood pressure ($\overline{P_A}$) and mean heart rate (f_H) for the seven animals (values are mean ± S.E.M.). The solid line displays the logistic function that best fits the experimental data. The broken line displays the predicted relationship at lower values of $\overline{P_A}$, where no experimental data could be obtained.

to emphasise that the baroreflex curve is saturated at high but not at low $\overline{P_A}$. Saturation occurs at $\overline{P_A}$ values above 9 kPa, where further decreases in f_H are minimal. However, irrespective of the dose of sodium nitroprusside employed, the minimal $\overline{P_A}$ values recorded averaged 5.0±0.4 kPa (mean ± S.E.M., N=7) (range 4.5–5.5 kPa). At this pressure, no saturation of the baroreflex response was observed, and the baroreflex curve is displayed with a broken line below 5 kPa (Fig. 2B) to indicate that no experimental data support the theoretical logistic model.

To gain a better understanding of the role of the baroreflex during hypotension, the responses to the highest doses of sodium nitroprusside (25 and 50 μg kg^{-1}) were reanalysed in detail. The initiation of ventilation was also considered since it was observed that ventilation always started shortly after the minimal pressure was achieved. The pattern observed was always similar (see example trace in Fig. 4) and can be described as follows: (1) a progressive decrease in $\overline{P_A}$ down to 5.0±0.4 kPa; (2) a delayed rise in f_H to a mean value of 27.1±6.5 beats min^{-1}; (3) initiation of inspiration with a single or multiple breaths, 52±31 s (mean ± S.E.M., N=5) after minimal $\overline{P_A}$; and (4) a further increase in f_H associated with the ventilation bout.

Table 2. *Values of the best fit to the four-variable sigmoidal model (see text for details), gain of the baroreflex and coefficient of determination for diastolic pressure, mean aortic pressure, systolic pressure and pulse pressure*

		P_D	$\overline{P_A}$	P_S	P_P
A	(beats min^{-1})	36.23	40.84	44.90	38.26
B	(beats min^{-1} kPa^{-1})	6.66	6.34	7.01	16.49
C	(kPa)	5.63	6.08	7.40	2.43
D	(beats min^{-1})	13.30	13.32	13.64	15.24
Gain	(beats min^{-1} kPa^{-1})	6.78	7.17	7.41	39.05
	(ms kPa^{-1})	663.62	586.68	518.53	3274.11
r^2		0.403	0.435	0.482	0.513

P_D, diastolic pressure; $\overline{P_A}$, mean aortic pressure; P_S, systolic pressure; P_P, pulse pressure.

Discussion

Critique of the method

The pharmacological approach has been the most common method of studying baroreflexes in non-mammalian vertebrates. Nevertheless, its major drawback is that only the cardiac limb of the baroreflex is evaluated because the method is based on altering peripheral resistance. At the same time, the pharmacological alteration of peripheral resistance can result in an overestimation of the sensitivity of the cardiac limb.

The two methodological alternatives to this method are the utilisation of inflatable balloons to occlude the vessels mechanically and thus to alter blood pressure (Berger *et al.* 1980) or the isolation of the baroreceptive areas using an open-loop arrangement (West and Van Vliet, 1983). Both methods require a precise knowledge of the location of the

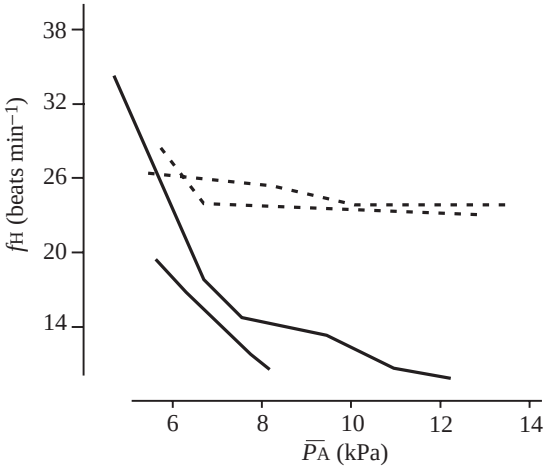

Fig. 3. Relationship between mean arterial blood pressure ($\overline{P_A}$) and heart rate (f_H) before and after complete autonomic blockade of the heart in two crocodiles. Solid lines, control; broken lines, after autonomic blockade.

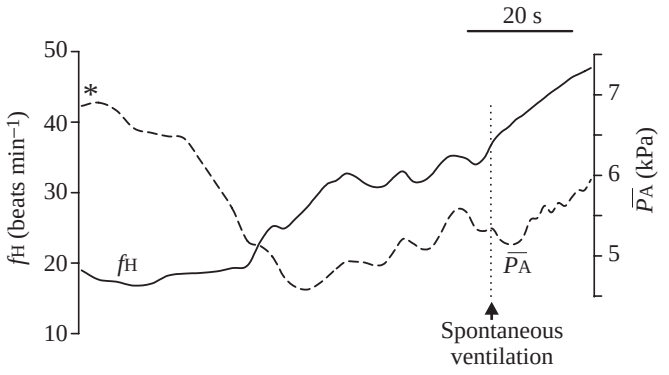

Fig. 4. Original recording of a baroreflex episode after injection of 25 μg kg^{-1} sodium nitroprusside (at the asterisk). Mean arterial blood pressure ($\overline{P}_A$), broken line; heart rate (f_H), continuous line. The onset of ventilation is marked by an upward-pointing arrow.

baroreceptive areas, which is lacking for crocodilians. In *Emys orbicularis* (a turtle) and *Trachydosaurus rugosus* (a lizard), the baroreceptor fibres arise from the proximal truncus (reviewed by Jones and Milsom, 1982; Berger, 1987) and a similar position has been described for two snake species (Backhouse *et al.* 1989). If the baroreceptive areas in crocodiles are located in the same position, a study of the baroreflex using an open-loop arrangement would be extremely complicated because the area cannot easily be isolated without occluding the entire outflow of the heart. On the other hand, the use of inflatable balloons would require extensive surgery and the achievement of high pressures is not guaranteed, as already pointed out (Berger, 1987). Aside from this, the pharmacological approach has the advantage of indiscriminate stimulation of the baroreceptors, which can be important because distinct baroreceptive areas are known in mammals (aortic arch and carotid sinuses) and amphibians (aortic arch, carotid arch and pulmocutaneous artery).

Smith *et al.* (1981) reported direct positive chronotropic effects with the use of phenylephrine and unreliable long-lasting effects of nitroprusside in the toad *Bufo marinus*. Studies on other amphibian species have not reported these problems (Millard and Moalli, 1980; Herman and Sandoval, 1983). However, with respect to sodium nitroprusside (no doses are reported for phenylephrine), the maximum dose employed by Smith *et al.* (1981) was more than 1000 times larger than the maximum dose used in our study (50 μg kg^{-1}).

Comparison of resting values and inter-individual differences

Resting heart rates and blood pressures in *C. porosus* are in agreement with previously published values. Wright *et al.* (1992) reported a mean heart rate of 23.5 beats min^{-1} (N=5), a value slightly above our mean value of 19.1 beats min^{-1}. Grigg and Johansen (1987) recorded a mean aortic pressure of 7.94 kPa in animals breathing on the surface (N=7), while Axelsson *et al.* (1997) reported a mean aortic pressure of 6.2 kPa. Our value in non-ventilating conditions was 7.1 kPa. Because of the reasonably long recovery period (36–48 h) and because the resting values were maintained throughout the 2 day experimental protocol, we are confident that our measurements reliably reflect the cardiovascular status of reasonably non-stressed animals.

The differences observed between individuals cannot be attributed to the experimental methodology or protocol. We are inclined to believe that the differences in heart rate and blood pressure arose from behavioural patterns associated with dominance/submission relationships in a well-known territorial species (Lang, 1989). Such differences could be seen when handling the animals during the normal experimental protocol: while some were docile and calm, others reacted aggressively without the least perturbation. In mice, psychosocial stress is known to induce hypertension in dominant individuals associated with enhanced adrenocortical activation and higher levels of glucocorticoids and catecholamines (Haemisch and Gärtner, 1997).

Characterization of the baroreflex

Despite the inter-individual differences, the $f_H/\overline{P}_A$ curve was similar for all the animals. The experimental data agree well with a hyperbolic curve limited by a f_H of 13 beats min^{-1} when pressure goes towards infinity and to a low $\overline{P}_A$ of 4 kPa when f_H goes towards infinity. Given that baroreceptor stimulation and the sinus node response display a sigmoidal relationship (Downing, 1979), the four-variable sigmoidal logistic function described by Reid (1996) was preferred even with no experimental support for a saturated response at low $\overline{P}_A$ values (dotted line in Fig. 2B). The coefficients of determination obtained were low, between 0.4 and 0.51, but again this is more a result of the high level of variation among animals than of the variation in baroreflex responses.

At the high $\overline{P}_A$ values achieved in response to phenylephrine infusion, the baroreflex is saturated at pressures above 9–10 kPa. From the maximum $\overline{P}_A$ values recorded in undisturbed animals (Grigg and Johansen, 1987; Axelsson *et al.* 1997), it appears that the effective range of baroreflex regulation coincides with the *in vivo* maximal pressures. Thus, the baroreflex constitutes the first line of defence against acute hypertensive episodes. In crocodilians, the impact of hypertension on the net filtration of plasma across the pulmonary capillaries might be attenuated in comparison with that in toads (West and Van Vliet, 1994) or turtles (Burggren, 1982) because of the almost completely divided circulation of crocodilians and the fact that pulmonary pressures are lower than systemic pressures in these animals. However, hypertension will still have an effect on coronary circulation and myocardial oxygenation as well as on the development of lesions in systemic vessels (Van Vliet and West, 1994).

The linear part of the baroreflex curve is a common trend in all the studies on the reptilian baroreflex (*Trachemys scripta* and *Chrysemys picta*, Stephens *et al.* 1983; *Trachemys scripta*, Millard and Moalli, 1980; *Trachydosaurus rugosus*, Berger *et al.* 1980). Such a linear relationship suggests a role for the baroreflex as a proportional controller (Jones and Milsom, 1982) (see Fig. 5).

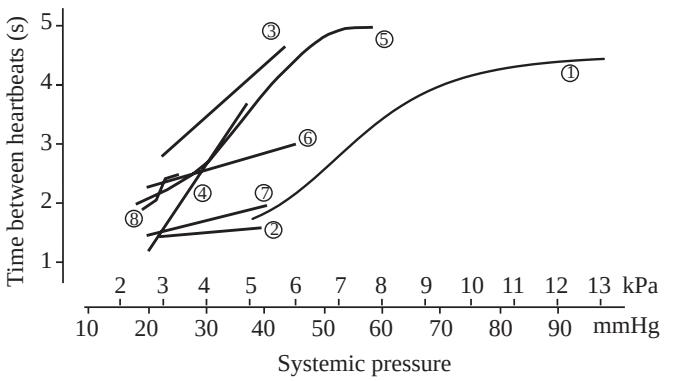

Fig. 5. Comparison of the relationship between systemic blood pressure and heart rate in different amphibians and reptiles. 1, *Crocodylus porosus* (present study); 2, *Trachemys scripta* and *Chrysemys picta* (Stephens *et al.* 1983); 3, *Trachemys scripta* (Millard and Moalli, 1980); 4, *Trachydosaurus rugosus* (Berger *et al.* 1980); 5, *Bufo marinus* (Van Vliet and West, 1989); 6, *Rana catesbeiana* (Millard and Moalli, 1980); 7, *Bufo marinus* (Smith *et al.* 1981); 8, *Rana catesbeiana* (Herman and Sandoval, 1983). See Table 3 for values of maximal gain of the baroreflex

Segura and Kacelnik (1977) report a differential component in blood pressure control in the lizard *Tupinambis rufescens*. This is based on the decrease in the ringing behaviour (the appearance of damped and irregular fluctuations in blood pressure when tegmental stimulation was removed) and a decrease in the steady error (time to return to control values after withdrawal of tegmental stimulation) in comparison with the toad *Bufo arenarum*. The operation of a differential controller in *C. porosus* is also supported by the analysis of the P_P/f_H relationship (Table 2). This analysis is complicated by the fact that P_P is positively correlated with $\overline{P_A}$, i.e. when $\overline{P_A}$ changed, P_P also changed proportionally ($\overline{P_A}/P_P$=3). After correcting the gain using this ratio, the relative gain for P_P is 13 beats min^{-1} kPa^{-1}, almost double the gain for $\overline{P_A}$ (7.17 beats min^{-1} kPa^{-1}). This, together with a higher coefficient of determination (0.513 for P_P and 0.435 for $\overline{P_A}$), is a strong, although indirect, indication of the differential component of the crocodilian baroreflex.

Maximal sensitivity was found at a $\overline{P_A}$ of 6.1 kPa, a value slightly below the resting value of 7.1 kPa. This is a good indication of the relevance of the baroreflex in cardiovascular regulation because it will be maximally active at pressures slightly below resting $\overline{P_A}$. This is the common situation in mammals such as the rabbit (Korner *et al.* 1972). Only an incomplete analysis has been made in other reptilian species, but it also appears to be the case in *Trachemys scripta* and *Trachydosaurus rugosus* (Table 3). This is in contrast with the toad *Bufo marinus*, where maximal gain is obtained at 3.9 kPa, while resting $\overline{P_A}$ is only 2.7 kPa. Van Vliet and West (1989) reason that baroreceptor endings in toads are designed to prevent pulmonary oedema by reacting to hypertensive episodes.

The reason behind this difference might be the presence or absence of myelinated afferent baroreceptor A fibres. While all vertebrates studied so far have unmyelinated C fibres, A fibres have only been positively identified in mammals (for a review, see Van Vliet and West, 1994). C fibres have higher thresholds and lower discharge rates than A fibres and are thought to be primarily antihypertensive (Thoren and Jones, 1977). In contrast, A fibres display low-pressure thresholds that would make them effective at normal pressures.

In nerve recording studies, no trace of A fibres has been found in toads (Van Vliet and West, 1987) or snakes (Backhouse *et al.* 1989). In contrast, Berger *et al.* (1982) report the presence of non-adrenergic myelinated nerve profiles in the truncus of *Trachydosaurus rugosus*, but their function was not studied. On the basis of the maximal baroreflex sensitivity at pressures below resting values, we can speculate that myelinated baroreceptor fibres are responsible for the

Table 3. *Interspecific comparison of baroreflex gain in different vertebrate species*

			$\overline{P_A}$				
Species	Maximum gain (ms kPa^{-1})	Normalized gain (%Δf_H kPa^{-1})	Maximum gain (kPa)	Resting (kPa)	Method	Model	Reference
Rana catesbeiana	288.8	13.2	2.4–4.5	2.9	Pharmacological	Linear	Millard and Moalli (1980)
Bufo marinus	178	13	–	4	Cuffs	–	Smith *et al.* (1981)
Bufo marinus	1027	39	3.9	2.7	Pharmacological	Third-order regression	Van Vliet and West (1989)
Trachemys scripta +*Chrysemys picta*	61.89	3.6	2.4–4.5	2.8	Pharmacological	Linear	Stephens *et al.* (1983)
Trachemys scripta	712	24	2.4–4.8	2.7	Pharmacological	Linear	Millard and Moalli (1980)
Trachydosaurus rugosus	1370	98	3–5	4.9	Cuffs	Linear	Berger *et al.* (1980)
Crocodylus porosus	586	47.6	6.1	7.1	Pharmacological	Logistic	This study
Anas boscas	15.8	4.9	5.9–21.6	16.6	Pharmacological	Linear	Millard (1980)
Rabbit	39	15	12.7	12.3	Cuffs	–	Korner *et al.* (1972)
Rabbit	58.8	22.5	8.7	–	Pharmacological	Logistic	Reid (1996)

hypotensive baroreflex in resting crocodiles, but a detailed study of the morphology and functional properties of baroreceptor fibres is required.

The marked reduction of baroreflex sensitivity following the administration of atropine and sotalol is in agreement with the well-known dependence of the baroreflex on the autonomic innervation of the heart. The slight increase in sensitivity at lower $\overline{P_A}$ values can be attributed to an incomplete adrenergic blockade with sotalol, because the effects of the drug wear off partially after 2–3 h. The effect appears only when pressures are decreased with sodium nitroprusside because the cardiac limb of the baroreflex during hypotension is mostly dependent on sympathetic activation (Korner, 1971).

Baroreflex and shunting

The baroreflex curve had a sharply defined minimum $\overline{P_A}$. Even with the highest doses of sodium nitroprusside (25 and 50 μg kg^{-1}), pressures below 4.5 kPa were not recorded (5.0±0.4 kPa was the mean value for the seven animals). It is well documented that systemic hypotension induces pulmonary-to-systemic shunting through the opening of the semilunar valves of the left aortic arch (Shelton and Jones, 1991). On a short-term basis, the onset of shunting may counteract hypotension by increasing blood volume in the systemic circulation. This can be demonstrated by comparing our minimal pressures with estimated threshold values from published recordings of right ventricular and left aortic pressures. In the alligator, the threshold pressure for shunt development is 4 kPa (White, 1969; Jones and Shelton, 1993) and it is 6 kPa in saltwater crocodiles (Grigg and Johansen, 1987). Additionally, Jones and Shelton (1993) showed that the frequency of shunting increased markedly in the range of systemic pressures of 4–6 kPa in undisturbed alligators.

Furthermore, hypotension induced by large doses of sodium nitroprusside was always accompanied by the onset of a ventilation bout. Since the ventilatory pattern in crocodilians is intermittent and irregular, the association between these phenomena (low $\overline{P_A}$ due to sodium nitroprusside infusion and onset of ventilation) could be coincidental, but we were able to reproduce the same effect repeatedly with the injection of the largest doses of sodium nitroprusside. The time lag between minimal pressure and the onset of ventilation was highly variable among individuals (52±31 s) but was long enough to exclude a direct effect, i.e. ventilation is not stimulated by the drop in systemic pressure *per se*. We suggest that the onset of the ventilation bout is triggered *via* chemoreflexes stimulated by a decrease in blood P_{O_2}. The drop in P_{O_2} could be caused by the impaired blood oxygenation associated with hypotension and the development of the pulmonary-to-systemic shunt (Douse and Mitchell, 1992), but we cannot provide direct experimental evidence for this.

The sequence of events after sodium nitroprusside infusion illustrates the synergistic effect of the baroreflex and shunting to buffer marked decreases in systemic pressure (Fig. 4): (1) a decrease in $\overline{P_A}$ resulting from sodium nitroprusside infusion; (2) mechanical control; shunt established when $\overline{P_A}$ reaches 5.0±0.4 kPa; (3) nervous control; f_H rises to a mean value of 27.1±6.5 beats min^{-1} caused by the baroreflex; (4) decreased P_{O_2} and (5) chemoreflex stimulation and the onset of ventilation.

The immediate effects of the shunt on blood pressure regulation could be physiologically significant only as an emergency back-up, its long-term effects blunted by the decrease in shunt flow as systemic pressure recovers. However, it is also clear that the baroreflex itself is sluggish in counteracting the pressure drop imposed artificially by sodium nitroprusside and that the further contribution of chemoreflexes is required.

Phylogeny of baroreflex regulation

In its overall sensitivity, the baroreflex appears to be better developed in mammals than in amphibians. The open-loop gain of the baroreflex (G_o) is a direct indicator of the total performance of the reflex in compensating for pressure changes, encompassing both the cardiac and the peripheral limb of the reflex (for a more extensive discussion, see Van Vliet and West, 1994). G_o values can be used to predict the performance of the system in the closed-loop state (G_c). G_c ranges from 0 (no compensation) to 1 (full compensation or 100 % efficiency). G_c values obtained in mammals range from 0.5 to 0.75, while the values obtained in toads are below 0.5. However, no open-loop studies have been carried out in reptiles or birds, and it might be premature to place too much reliance on this conclusion.

Bagshaw (1985) argues that the baroreflex has shifted from a primarily f_H-based control mechanism to a peripheral-resistance-based mechanism. This being true, the relative contribution of the cardiac limb of the reflex would be smaller in mammals than in reptiles or amphibians. This hypothesis is appealing because it fits with the overall trend from cholinergic to adrenergic sympathetic control of the vascular system during evolution (Bagshaw, 1985), but it is difficult to reconcile with the empirical results. The normalised maximal reflex gain in *C. porosus* is at least twice as high the sensitivity as in any other vertebrate except the lizard *T. rugosus* (Table 3), which would put reptiles (except turtles) at the top of the vertebrate groups in terms of baroreflex sensitivity.

In conclusion, very little information can be extrapolated from the baroreflex alone to further understand the phylogeny of cardiovascular control. Rather than being surprised, we see this as a clear indication of how complex it is to determine phylogenetic trends when many different elements of the system under study are evolving simultaneously; for example, in the cardiovascular system (a) the degree of separation of the cardiac chambers, (b) the prevalence of sympathetic *versus* parasympathetic control of pacemaker areas, (c) the extension of the sympathetic innervation of the ventricle and peripheral vessels or (d) the location and sensitivity of sensory afferent elements. All these components change markedly between the different vertebrate groups, and it will be their overall performance that will drive common evolution towards a more refined system.

The project was funded by a small Australian Research Grant to C.E.F. J.A. was a recipient of a postdoctoral fellowship from the American Heart Association Nevada Affiliate (9504504S). M.A. acknowledges the Swedish Forestry and Agricultural Research Council and the Knut and Alice Wallenberg Foundation.

References

ALTMAN, P. L. AND DITTMER, D. S. (1971). Respiration and circulation. In *Biological Handbooks*. Bethesda: Federation of American Societies for Experimental Biology.

AXELSSON, M. AND FRANKLIN, C. E. (1997). From anatomy to angioscopy, 163 years of crocodilian cardiovascular research. *Comp. Biochem. Physiol.* **118**, 51–62.

AXELSSON, M., FRANKLIN, C. E., FRITSCHE, R., GRIGG, G. C. AND NILSSON, S. (1997). The subpulmonary conus and the arterial anastomosis as important sites of cardiovascular regulation in the crocodile *Crocodylus porosus*. *J. exp. Biol.* **200**, 807–814.

BACKHOUSE, S. S., HARPER, A. A., VAN VLIET, B. N. AND WEST, N. H. (1989). Localization and conduction velocities of arterial baroreceptors in the anaesthetized snake. *J. Physiol., Lond.* **418**, 135P.

BAGSHAW, R. J. (1985). Evolution of cardiovascular baroreceptor control. *Biol. Rev.* **60**, 121–162.

BERGER, P. J. (1987). The reptilian baroreceptor and its role in cardiovascular control. *Am. Zool.* **27**, 111–120.

BERGER, P. J., EVANS, B. K. AND SMITH, D. G. (1980). Localization of baroreceptors and gain of the baroreceptor–heart rate reflex in the lizard *Trachydosaurus rugosus*. *J. exp. Biol.* **86**, 197–209.

BERGER, P. J., GIBBINS, I. L., HARDS, D. K. AND CROSBY, L. J. (1982). The distribution and ultrastructure of sensory elements in the baroreceptor region of the truncus arteriosus of the lizard *Trachysaurus rugosus*. *Cell. Tissue Res.* **226**, 389–406.

BURGGREN, W. W. (1982). Pulmonary blood plasma filtration in reptiles: a 'wet' vertebrate lung? *Science* **215**, 77–78.

DOUSE, M. A. AND MITCHELL, G. S. (1992). Episodic breathing in alligators: role of sensory feedback. *Respir. Physiol.* **87**, 77–90.

DOWNING, S. E. (1979). Baroreceptor regulation of the heart. In *Handbook of Physiology. The Cardiovascular System. I. The Heart* (ed. R. M. Berne and N. Sperelakis), pp. 621–652. Bethesda: American Physiological Society.

GRIGG, G. C. AND JOHANSEN, K. (1987). Cardiovascular dynamics in *Crocodylus porosus* breathing air and during voluntary aerobic dives. *J. comp. Physiol.* **157**, 381–392.

GUIBÉ, J. (1970). La musculature. In *Traité de Zoologie. Anatomie, Systématique, Biologie. Reptiles. Caracteres Généraux et Anatomie*, vol. XIV, fascicule II (ed. P.-P. Grassé), pp. 144–180: Paris: Masson et Cie.

HAEMISCH, A. AND GÄRTNER, K. (1997). Effects of cage enrichment on territorial aggression and stress physiology in male laboratory mice. *Acta physiol. scand.* **161**, 73–76.

HERMAN, C. A. AND SANDOVAL, E. J. (1983). Catecholamine effects on blood pressure and heart rate in the American bullfrog, *Rana catesbeiana*. *Gen. comp. Endocr.* **52**, 142–148.

HUGGINS, S. E., HOFF, H. E. AND PENA, R. V. (1970). The respiratory–heart rate response in crocodilian reptiles. *Physiol. Zool.* **43**, 10–18.

JONES, D. R. AND MILSOM, W. K. (1982). Peripheral receptors affecting breathing and cardiovascular function in non-mammalian vertebrates. *J. exp. Biol.* **100**, 59–91.

JONES, D. R. AND SHELTON, G. (1993). The physiology of the alligator heart: left aortic flow patterns and right-to-left shunts. *J. exp. Biol.* **176**, 247–269.

KORNER, P. I. (1971). Integrative neural cardiovascular control. *Physiol. Rev.* **51**, 312–367.

KORNER, P. I., SHAW, J., WEST, M. J. AND OLIVER, J. R. (1972). Central nervous system control of baroreceptor reflexes in the rabbit. *Circulation Res.* **31**, 637–652.

LANG, J. W. (1989). Social behaviour. In *Crocodiles and Alligators* (ed. C. A. Ross), pp. 102–117. Sydney: Golden Press.

MILLARD, R. W. (1980). Depressed baroreceptor–cardiac reflex sensitivity during simulated diving in ducks. *Comp. Biochem. Physiol.* **65**A, 247–249.

MILLARD, R. W. AND MOALLI, R. (1980). Baroreflex sensitivity in an amphibian, *Rana catesbeiana* and a reptilian, *Pseudemys scripta elegans*. *J. exp. Zool.* **213**, 283–288.

REID, I. A. (1996). Angiotensin II and baroreflex control of heart rate. *News physiol. Sci.* **11**, 270–274.

SEGURA, E. T. AND KACELNIK, A. (1977). Cardiorespiratory and EEG responses to the stimulation of the mesencephalic tegmentum in toads, lizards and rats. *Exp. Neurol.* **57**, 364–373.

SHELTON, G. AND JONES, D. R. (1991). The physiology of the alligator heart: the cardiac cycle. *J. exp. Biol.* **158**, 539–564.

SMITH, D. G., BERGER, P. J. AND EVANS, B. K. (1981). Baroreceptor control of heart rate in the conscious toad *Bufo marinus*. *Am. J. Physiol.* **241**, R307–R311.

SOKAL, R. R. AND ROHLF, F. J. (1995). *Biometry: the Principles and Practice of Statistics in Biological Research*. New York: W. H. Freeman.

STEPHENS, G. A., SHIRER, H. W., TRANK, J. W. AND GOETZ, K. L. (1983). Arterial baroreceptor reflex control of heart rate in two species of turtle. *Am. J. Physiol.* **244**, R544–R552.

THOREN, P. AND JONES, J. V. (1977). Characteristics of aortic baroreceptor C-fibres in the rabbit. *Acta physiol. scand.* **99**, 448–456.

VAN VLIET, B. N. AND WEST, N. H. (1987). Responses to circulatory pressures and conduction velocity, of pulmocutaneous baroreceptors in *Bufo marinus*. *J. Physiol., Lond.* **388**, 41–53.

VAN VLIET, B. N. AND WEST, N. H. (1989). Cardiovascular responses to denervation of pulmocutaneous baroreceptors in toads. *Am. J. Physiol.* **256**, R946–R954.

VAN VLIET, B. N. AND WEST, N. H. (1994). Phylogenetic trends in the baroreceptor control of arterial blood pressure. *Physiol. Zool.* **67**, 1284–1304.

WEST, N. H. AND VAN VLIET, B. N. (1983). Open-loop analysis of the pulmocutaneous baroreflex in the toad *Bufo marinus*. *Am. J. Physiol.* **245**, R642–R650.

WEST, N. H. AND VAN VLIET, B. N. (1994). The role of arterial baroreceptors in the undivided circulation of anuran amphibians. *Physiol. Zool.* **67**, 1305–1324.

WHITE, F. N. (1969). Redistribution of cardiac output in the diving alligator. *Copeia* **1969**, 567–570.

WRIGHT, J. C., GRIGG, G. C. AND FRANKLIN, C. E. (1992). Redistribution of air within the lungs may potentiate 'fright' bradycardia in submerged crocodiles (*Crocodylus porosus*). *Comp. Biochem. Physiol.* A **102**, 33–36.