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Ontogeny of baroreflex control in the American alligator *Alligator mississippiensis*

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Summary

Baroreflex regulation appears in different species at different points in embryonic development. This study was designed to understand the development of the baroreflex in embryos of the American alligator at four different points of embryonic development (60%, 70%, 80% and 90% of a total incubation period of 72 days) and in 1-week-old hatchlings. Data from a separate study on 1-year-old alligators were included for comparison. The gain of the cardiac limb of the baroreflex was calculated from heart rate changes triggered by pharmacological manipulation of arterial pressure with sodium nitroprusside and phenylephrine. The results demonstrated that a vagally mediated hypertensive baroreflex was present during the final 30% of alligator development. A hypotensive baroreflex was not present in embryos but appeared in hatchlings, mediated by a combined effect of vagal and sympathetic efferents. Absolute baroreflex gain was maximal at 80% of incubation (41.22 beats kPa^{-1} min^{-1}) and dropped thereafter, reaching a minimum in 1-year-old alligators (9.69 beats kPa^{-1} min^{-1}). When the baroreflex gain was normalized to resting arterial pressure and heart rate, the maximum gain was observed in 1-year-old alligators (normalized index of 2.12 *versus* 0.75 in hatchlings and 0.69 as the highest gain in embryos). In conclusion, baroreflex regulation appeared during embryonic development with a substantial gain. These findings indicate that embryonic development is a period of preparation for cardiovascular regulatory mechanisms that will be necessary in adult life and that the baroreflex control mechanism is required for cardiovascular control during ontogeny.

Key words: baroreflex regulation, embryonic development, baroreflex gain, cardiovascular regulation, American alligator, *Alligator mississippiensis*.

Introduction

The baroreflex, or pressure reflex, is the primary mechanism in adult vertebrates for rapid regulation of arterial pressure through changes in heart rate and peripheral resistance. Such a mechanism is important for buffering fluctuations in arterial pressure, to maintain tissue perfusion pressure and, ultimately, to supply metabolic fuels to the end-organs (Van Vliet and West, 1994). While the functional significance of the baroreflex is clear in adult organisms, the importance of this reflex during embryonic or fetal development is less clarified.

The two primary species used to study vertebrate cardiovascular development, sheep and chickens, both possess a functional baroreflex during fetal or embryonic life (Blanco et al., 1988; Segar, 1992; Altimiras and Crossley, 2000). This suggests that either the baroreflex is an important component of cardiovascular regulation during development or that it simply becomes functional in anticipation of its neonatal utility. Work in embryonic chickens suggests the latter, given that barostatic reflexes emerge very late in development and have a lower gain compared with those in adults (Altimiras and Crossley, 2000). By contrast, fetal sheep possess a baroreflex as early as 60% of gestation, with a decrease in sensitivity and an increasing set point during development (Blanco et al., 1988; Segar, 1997; Segar et al., 1992). This apparent contradiction between a mammalian fetus and an avian embryo may be the result of differing gestational conditions. While the sheep develops *in utero*, the chicken develops independent of the mother in an egg case. The delayed baroreflex maturation in chickens could therefore be a particular feature of development within an egg case.

To further understand the relevance of an embryonic baroreflex, we chose to study it in embryos of the American alligator. American alligators develop within an egg case, similar to the chicken embryo, thus excluding embryonic maternal physiological interactions. While this is a characteristic shared with birds, reptile eggs exhibit a greater permeability to water flux, possibly subjecting the developing animal to periods of dehydration that might require the earlier onset of a cardiovascular regulatory mechanism. Thus, this

study was conducted in embryos of the American alligators to determine the time of activation and the magnitude of the cardiac limb of the baroreflex.

Materials and methods

60 newly laid eggs from 10 clutches of the American alligator (*Alligator mississippiensis* Daudin) were collected from field nests in the Rockefeller Wildlife Refuge at Grand Chenier, LA, USA. At approximately 10 days of incubation (total incubation period=72 days), all eggs were transported by air to the Department of Ecology and Evolutionary Biology at the University of California at Irvine. Upon arrival, eggs were numbered, weighed and placed in plastic containers containing vermiculite mixed with water in a 2:1 ratio, with eggs of each clutch randomly distributed between the boxes. All egg boxes were maintained at 30°C in an environmental chamber during the course of the study. To ensure that the water content of the vermiculite was maintained, each egg box was weighed at the onset of the experiment and twice weekly afterwards, with water continuously added as needed. Eggs were removed from incubation for study at 60%, 70%, 80% and 90% of the 72-day total incubation period. In addition, six animals (from six different clutches) were hatched to study baroreflex responses at 1 week of age.

Surgical procedure in embryos

An egg at the appropriate time of incubation was removed from the incubator, candled for the location of a tertiary chorioallantoic artery and a tertiary chorioallantoic vein and placed in a water-jacketed chamber at 30°C. The chamber was open at the top to allow the manipulation of the egg during surgery. A 1.5 cm×1.5 cm window was cut in the shell, exposing the underlying chorioallantoic vessels. The previously located artery and vein were then isolated and catheterized using heat-pulled polyethylene tube (PE-90), as previously described (Altimiras and Crossley, 2000). Following catheterization, the egg was placed in a thermostatically controlled glass chamber that was fitted with a lid with multiple ports to externalize catheters. The venous catheter was used for the injection of drugs, while the arterial catheter was used for pressure measurements.

Surgical procedure in 1-week-old hatchlings

Additional surgical procedures were conducted on six 1-week-old American alligators. Each animal was placed in a plastic box containing a cloth saturated with isoflurane to induce anesthesia. Once anesthetized, the glottis was intubated with PE-90 tubing and ventilated (SAR-830 ventilator) at 2 breaths min^{-1} (tidal volume=1 ml) with a 2% isoflurane/room air mixture using a FluTec vaporizer (OH, USA) until the surgical plane of anesthesia was achieved. A 1 cm cut was made in the skin at the midline of the dorsal surface of a rear limb above the femur, the skin was retracted and underlying musculature separated to expose the femoral artery and vein. Once isolated, both vessels were occlusively catheterized using heparinized saline-filled heat-pulled PE-50 tubing. Both catheters were then tunneled under the skin, externalized and fixed with a single suture to the back of the animal. A blind saline-filled injection port was connected to the end of the arterial catheter, and the venous catheter was heat-sealed. The skin incision was sutured closed with 4-0 suture and sealed with Vet-bond™ tissue adhesive. Once surgical procedures were completed, animals were placed in a 30 l glass aquarium covered with cardboard, with 1 cm of water in the bottom and maintained at 28°C. A saline-filled section of PE-90 tubing fitted with a 21-gauge needle was connected to the catheter port. This section of PE-90 tubing was connected to a pressure transducer (DP6100; Peter von Berg, Sweden) calibrated against a static column of water. All animals were allowed 24 h to recover prior to experimentation.

Signal recording and calibration

The arterial catheter was attached to a pressure transducer connected to a 4CHAMP amplifier (Somedic AB, Sweden) and the pressure trace stored in a DELL Latitude computer using a custom-made data acquisition program (LabView; National Instruments, USA). In all cases, reference zero pressure was set at the top of the experimental bath, and all values were corrected *a posteriori* to take into account the position of the heart as previously described (Altimiras and Crossley, 2000). In the hatchling alligators, the experimental zero was set at the level of the heart of the animal.

Experimental protocol

The measured hemodynamic variables were allowed to stabilize for 1 h to establish control values prior to any experimental manipulation. During this period, arterial pressure was recorded and heart rate was determined from the pressure trace with an online software tachograph.

After the control period, a pharmacological assessment of the cardiac limb of the baroreflex was carried out as previously described (Altimiras et al., 1998; Altimiras and Crossley, 2000). To assess the baroreflex response to a lowering of systemic pressure (hypotensive reflex), three sequential doses (25 μg kg^{-1}, 50 μg kg^{-1} and 100 μg kg^{-1}) of sodium nitroprusside (SNP) were injected followed by a saline flush. The total volume injected (75 μl, 105 μl, 105 μl and 150 μl to 60%, 70%, 80% and 90% embryos, respectively) was less than 5% of the embryonic blood volume estimated from data in chicken embryos. Previous tests showed that saline injections of up to 5% estimated blood volume did not elicit any pressure changes. Heart rate and blood pressure were allowed to return to control levels after each injection. After the third injection of SNP, the baroreflex response to an increased blood pressure (hypertensive reflex) was studied with three sequential doses (30 μg kg^{-1}, 60 μg kg^{-1} and 120 μg kg^{-1}) of phenylephrine (PE). Cardiovascular variables were also allowed to return to control levels between each dose. These protocols were conducted on embryos at 60% (N=7), 70% (N=8), 80% (N=9) and 90% of incubation (N=9) and in 1-week-old hatchling animals (N=6).

After completing this protocol, a single bolus of the muscarinic antagonist atropine (3 mg kg^{-1}) was injected to block any contribution of the parasympathetic autonomic nervous system to baroreflex regulation, and the baroreceptors were stimulated again with the largest dose of PE (120 μg kg^{-1}). At the conclusion of the experimental protocols, embryos were euthanized with an intravenous injection of sodium pentobarbital (50 mg kg^{-1}).

Calculations and statistics

The Wilcoxon nonparametric test was used to assess the response to different drug administrations (atropine, SNP and PE). A Mann–Whitney nonparametric *U*-test was used to determine differences through development. Since repeated tests were carried out, thereby using the same data more than once, the fiduciary limit (P=0.05) was corrected according to the number of times each data set was used, commonly 2–3 times since the tests between developmental intervals were restricted to adjacent days (thereby comparing 60% to 70%, 70% to 80%, 70% to 90% and 80% to 90%).

Baroreflex gain in embryos was calculated from the heart-rate–arterial-pressure linear slope as reported previously (Altimiras and Crossley, 2000). The hatchling data was fitted to a four-variable sigmoid logistic function as previously described (Altimiras et al., 1998). Data from another study on 1-year-old alligators (B. Bagatto, D. A. Crossley, J. Altimiras and J. W. Hicks, unpublished) was fitted to the same type of model for comparison purposes. Although the baroreflex quantification methods differ between embryos and hatchlings, gain will be referred to as maximal gain in all cases (G_{50}; see Altimiras et al., 1998 for a more extended description).

To establish meaningful comparisons between different developmental stages, gain was normalized for control mean arterial pressure ($\overline{P}$a) and heart rate (f_H) similar to what was previously suggested (Berger et al., 1980). Normalized gain ($G_{50,norm}$; unitless) was calculated as: $G_{50,norm}=G_{50}(\overline{P}a/f_H)$.

$\overline{P}$a where baroreflex gain is maximal is also obtained from the sigmoid logistic model and will be reported as $\overline{P}a_{G50}$ (kPa). Data are shown as means ± S.E.M. Significant differences were all taken at the fiduciary level of P<0.05.

Results

Embryonic alligators exhibited a relatively constant heart rate (85±2 beats min^{-1}) during the final 40% of incubation (Fig. 1), but $\overline{P}$a increased by 3.5 times from 0.56±0.1 kPa to 2.0±0.15 kPa. After hatching, f_H fell to 32±2 beats min^{-1} while $\overline{P}$a continued to rise to 2.9±0.2 kPa (Fig. 1).

Pharmacological pressor responses

SNP induced a pronounced hypotension at all incubation ages (Fig. 2A). The magnitude of this response varied with drug dose and developmental age. The intermediate (50 μg kg^{-1}) and maximal (100 μg kg^{-1}) doses produced the greatest reduction in pressure at all incubation ages with a maximum at 80% of development ($\overline{P}$a dropped by 0.52±0.08 kPa).

PE injection produced a marked hypertensive response that was not dose dependent. While the maximal response to each drug dosage was the same, the effects of PE increased with progressive developmental age (Fig. 2B).

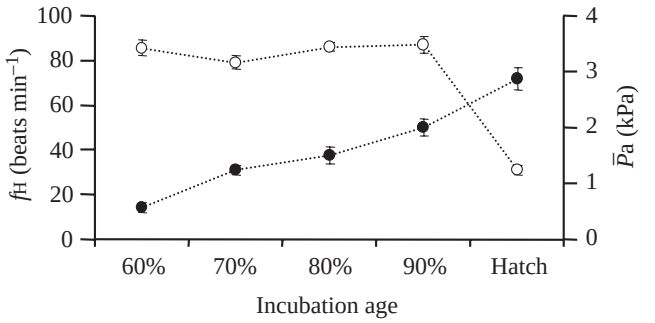

Fig. 1. Heart rate (f_H; open circles) and mean arterial pressure ($\overline{P}$a; closed circles) during development in the alligator. Data are means ± S.E.M. (N=7 at 60%, N=8 at 70%, N=8 at 80%, N=9 at 90% and N=6 for hatchlings).

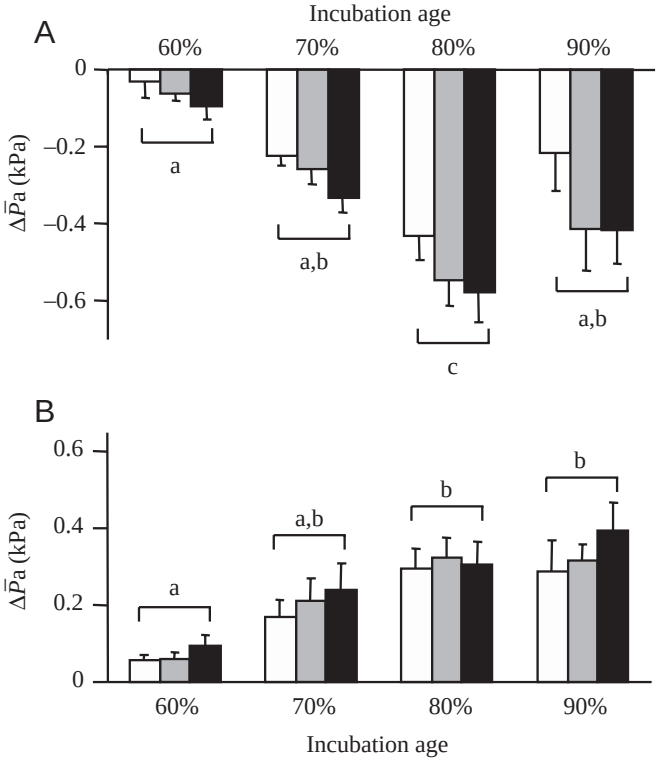

Fig. 2. Blood pressure changes ($\Delta\overline{P}$a) elicited by different doses of (A) sodium nitroprusside (SNP) and (B) phenylephrine (PE) at different ages of development. Open bars: 25 μg kg^{-1} SNP and 30 μg kg^{-1} PE, respectively; shaded bars: 50 μg kg^{-1} SNP and 60 μg kg^{-1} PE, respectively; closed bars: 100 μg kg^{-1} SNP and 120 μg kg^{-1} PE, respectively. Data are means ± S.E.M. (N=8). Dissimilar letters indicate differences between ages. All doses except the lowest dose of SNP at 60% of development induced significant changes in pressure.

Baroreflex assessment

Increased arterial pressure due to PE injection induced a transient reciprocal change in f_H (Fig. 3). This response is indicative of a functional baroreflex at 70% of embryonic alligator development and later. However, no hypotensive baroreflex response (increase in f_H) was observed after SNP injection at any embryonic age. Note, however, that the hypertensive heart rate response at 70% and 80% was brief and subsided even though $\overline{P}a$ continued to rise (Fig. 3). The mean time between the maximal heart rate drop elicited by the baroreflex and the maximal pressure response elicited by PE was 73±16 s at 70% and 47±14 s at 80%. This time was significantly decreased at 90% (9±7 s; $P<0.05$) because the heart rate response lasted longer.

The mean baroreflex response in embryonic alligators is shown in Fig. 4 by plotting the changes in f_H induced after pharmacological alteration of $\overline{P}a$. The highest baroreflex gain in response to increased $\overline{P}a$ was obtained at 80% of incubation.

In hatchlings, the baroreflex was responsive to hypotension as well as to hypertension (Fig. 5). The operational point of the reflex was shifted towards higher blood pressures and lower heart rates, and this was further shown in 1-year-old alligators (Fig. 6). For comparative purposes, Fig. 6 shows the $\overline{P}a$–f_H relationship at all ages studied. The operational point of the baroreflex together with maximal and normalized baroreflex gains are summarized in Table 1. As $\overline{P}a$ rose from 1.2±0.1 kPa (70%) to 4.3±0.1 kPa (1 year), G_{50} peaked at 80% of incubation (41.2 beats kPa^{-1} min^{-1}) and fell during the final

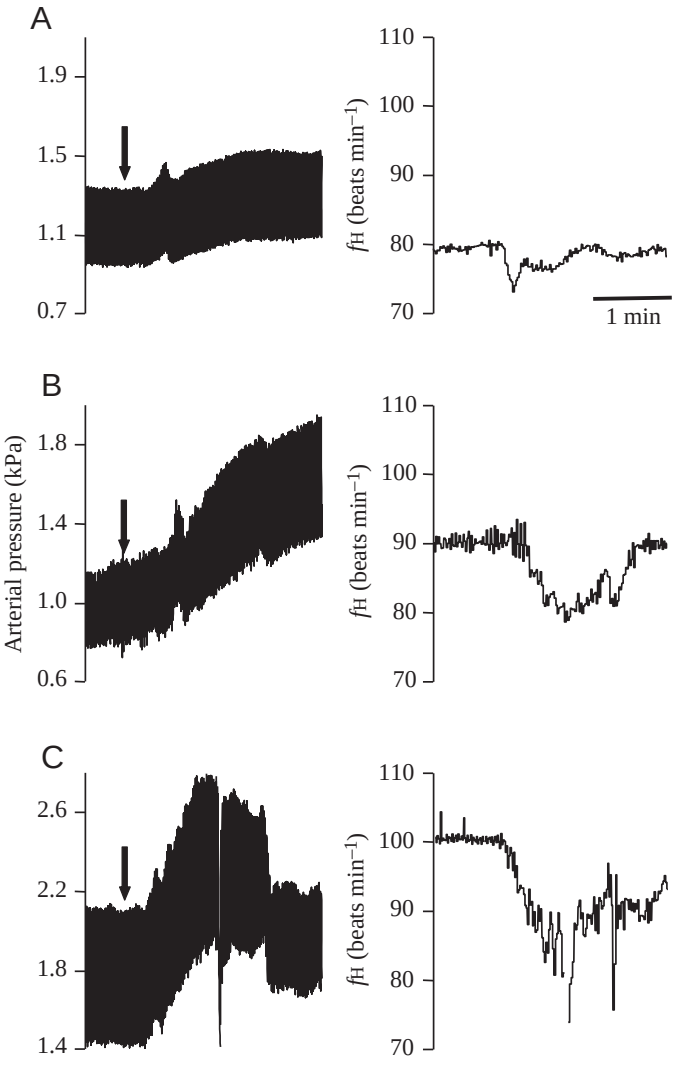

Fig. 3. Representative hypertensive baroreflex responses at (A) 70%, (B) 80% and (C) 90% of development after an injection of 120 μg kg^{-1} of phenylephrine (the highest dose). The time of injection is indicated by the arrow. While the *y*-axis absolute scales vary between developmental ages, the relative scales are maintained for comparative purposes. $\overline{P}a$, arterial pressure; f_H, heart rate.

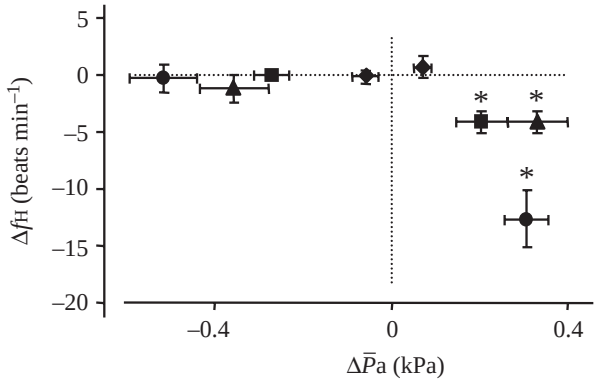

Fig. 4. The mean baroreflex responses at 60% (diamonds), 70% (squares), 80% (circles) and 90% (triangles) of development. Notice that decreases in blood pressure ($\overline{P}a$) with sodium nitroprusside (SNP) did not induce reciprocal changes in heart rate (f_H). Data are means ± S.E.M. (N=8). Asterisks indicate a significant heart rate response ($P<0.05$).

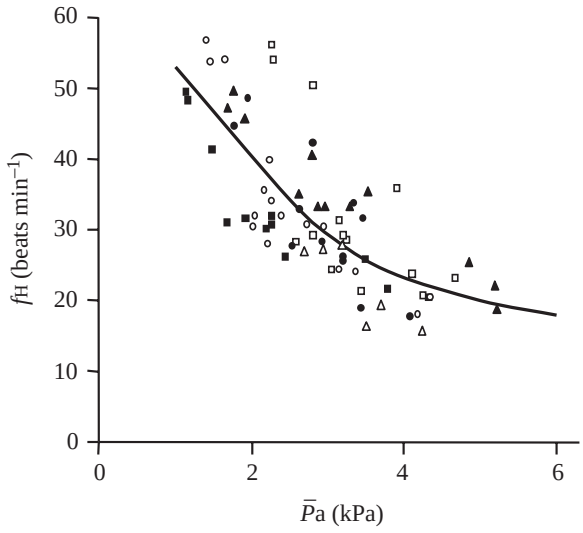

Fig. 5. Heart rate–mean blood pressure (f_H–$\overline{P}a$) relationship in 1-week-old hatchling alligators. The data from all pharmacological manipulations are combined in a single trace (N=6); different symbols represent different animals.

Table 1. *Features of the baroreflex at different developmental ages*

Age	Type	G_{50} (beats kPa min^{-1})	$G_{50,norm}$ (no units)	Resting $\bar{P}$a (kPa)	$\bar{P}a_{G50}$ (kPa)
70%	Hypertensive	20.25	0.33	1.24	–
80%	Hypertensive	41.22	0.69	1.49	–
90%	Hypertensive	12.54	0.29	2.01	–
1 week	Complete	11.46	0.75	2.87	2.34
1 year	Complete	9.69	2.12	4.3	4.06

Type indicates whether the baroreflex was active when pressure was increased (hypertensive) or when pressure was increased and decreased (complete). G_{50}, maximal baroreflex gain; $G_{50,norm}$, normalized maximal baroreflex gain; resting $\bar{P}$a, resting mean arterial pressure; $\bar{P}a_{G50}$, mean arterial pressure at G_{50}. $\bar{P}a_{G50}$ could not be calculated in embryos because the data were not fitted to a sigmoidal baroreflex model. See Materials and methods for further details.

20% of incubation (G_{50}=12.54 beats kPa^{-1} min^{-1} at 90%; Table 1). The gain of the baroreflex continued to fall in hatchlings, reaching a minimal level in 1-year-old animals (G_{50}=9.69 beats kPa^{-1} min^{-1}). Due to the different operational values for heart rate and blood pressure in embryos (high heart rates, low blood pressures) *versus* hatchlings (lower heart rates and higher blood pressures), normalized baroreflex gain ($G_{50,norm}$) was maximal in 1-year-old alligators (2.12%Δf_H %$\bar{P}$a^{-1}; Table 1).

Role of muscarinic receptors in baroreflex regulation

Given that the cardiac baroreflex response in adult crocodiles and all vertebrate species studied is largely dependent on vagal nerve activity (Altimiras et al., 1998), the experimental protocol was extended to verify this dependence by blocking muscarinic cholinergic receptors pharmacologically with atropine. The suitability of atropine (3 mg kg^{-1}) as a muscarinic antagonist was first checked by injecting a bolus dose of acetylcholine (100 μg kg^{-1}) before and after atropine blockade. The bradycardia elicited by acetylcholine was abolished by atropine injection in 70% and 90% embryos (Fig. 7).

After atropine injection, SNP and PE triggered the expected responses in blood pressure in embryos (Fig. 8A) and hatchlings (Fig. 9A), but reflexive f_H changes were largely blunted. The hypertensive reflex was abolished in 80% and 90% embryos (Fig. 8B) and also in hatchlings (Fig. 9B). Notice, however, that the hypotensive reflex tested with SNP was not completely abolished in hatchlings after atropine (Fig. 9B).

Discussion

This study was conducted in embryos of the American alligator to determine the time of activation and the magnitude of the cardiac limb of the baroreflex. Baroreflex function was tested through pharmacological manipulations of arterial

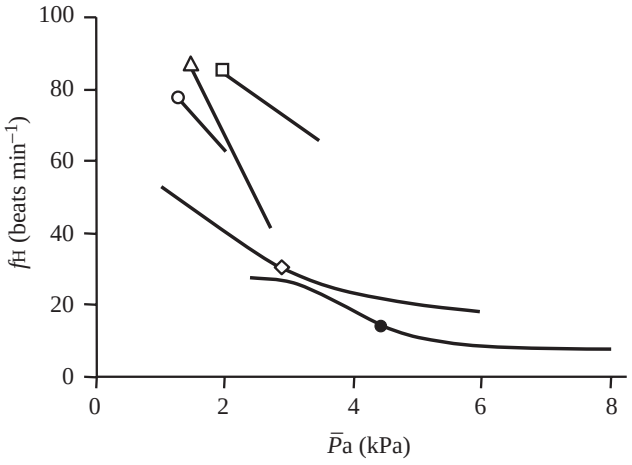

Fig. 6. Comparison of the heart rate–mean blood pressure (f_H–$\bar{P}$a) relationship for all developmental ages. The point in each trace indicates resting values for heart rate and mean arterial pressure: open circle, 70%; triangle, 80%; square, 90%; diamond, hatchling; filled circle, 1-year-old.

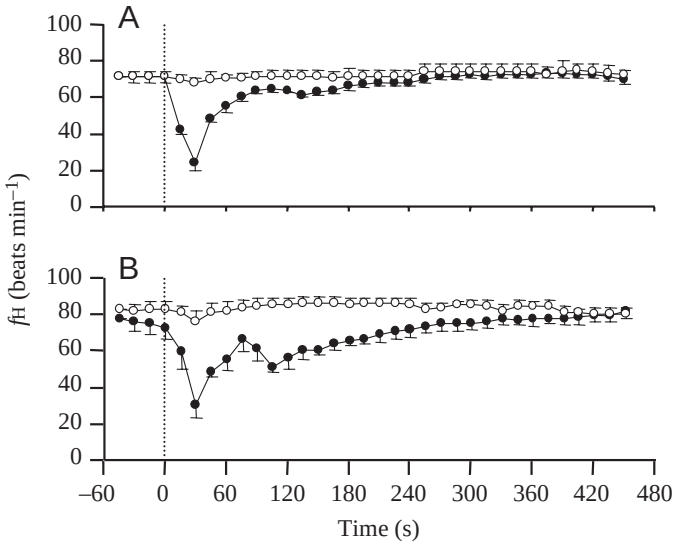

Fig. 7. Effect on heart rate (f_H) of a bolus injection of acetylcholine (100 μg kg^{-1}; time of injection indicated by dotted line) before (solid symbols) and after (open symbols) treatment with atropine (3 mg kg^{-1}) at 70% (A; N=4) and 90% (B; N=5) of development. Data are means ± S.E.M.

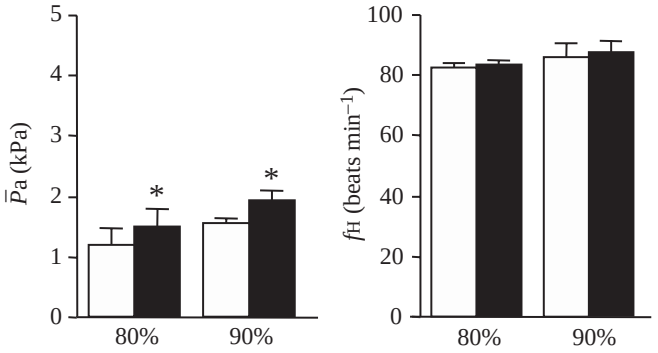

Fig. 8. Chorioallantoic membrane mean arterial pressure ($\bar{P}$a) and heart rate (f_H) before (open bars) and after (closed bars) injection of phenylephrine (120 μg kg^{-1}) in 80% and 90% embryos pre-treated with atropine (3 mg kg^{-1}). Notice that phenylephrine has significant effects on pressure but no effects on heart rate. Data are means ± S.E.M. (N=6). Asterisks indicate a significant response to the drug (P<0.05).

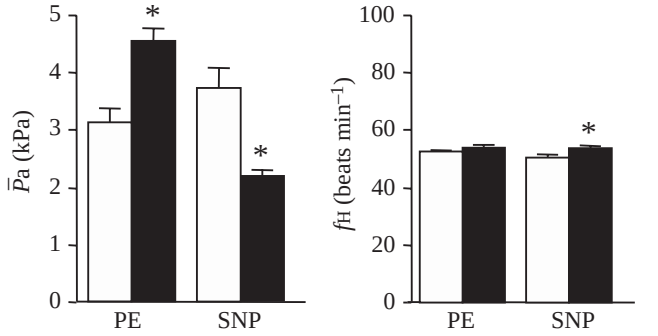

Fig. 9. Femoral arterial pressure and heart rate before (open bars) and after (closed bars) injection of phenylephrine (PE; 120 μg kg^{-1}) and sodium nitroprusside (SNP; 100 μg kg^{-1}) in hatchlings pre-treated with atropine (3 mg kg^{-1}). Data are means ± S.E.M. (N=6). Asterisks indicate a significant response to the drug (P<0.05).

pressure from 60% of incubation time to 1-week-old hatchling animals. The evidence obtained indicates that a vagally mediated hypertensive baroreflex was present during the final 30% of ontogeny. The hypotensive baroreflex, which was absent in embryos, appeared in hatchlings and was mediated by vagal efferents, with a minor contribution of sympathetic efferent mechanisms. Overall, these results indicate that the cardiac limb of the baroreflex matures during the last 40% of development. During this developmental period it undergoes changes in absolute and relative baroreflex gain into the first week of post-hatching life, suggesting an important role in embryonic cardiovascular regulation.

Developmental pattern of heart rate and mean arterial pressure

This study comprised the first assessment of basal blood pressure and heart rate during embryonic development in a crocodilian species. $\bar{P}$a rose consistently during the last 40% of incubation and continued to rise in hatchlings (Fig. 1), a pattern of pressure change that is consistent with that of other developing vertebrates (Blanco et al., 1988; Segar et al., 1992; Tazawa and Hou, 1997). By contrast, f_H remained constant during embryonic incubation and dropped markedly following hatching (Fig. 1). This sudden reduction in resting f_H is due, in part, to the onset of tonic vagal depression of heart rate in hatchling alligators (J. Altimiras and D. A. Crossley, II, unpublished observations). This pattern of f_H change is unique among the egg-laying species that have been studied thus far. In chicken embryos, for example, f_H is maintained at high levels post-hatching (reviewed in Tazawa and Hou, 1997). Pharmacological manipulation of the vasculature also revealed unique characteristics during alligator development.

Vascular response to sodium nitroprusside and phenylephrine

Embryonic alligators exhibited the typical 'adult' vascular responses to SNP, a receptor-independent vasodilator, and PE, a receptor-dependent vasoconstrictor. The vascular response to SNP was dose dependent at all developmental ages with maximal responses occurring at 80% of incubation (Fig. 2), which corresponds to the expected greatest vascular density based on embryonic chicken data (Romanoff, 1967). In contrast to the dose-dependent SNP response, PE induced a vasoconstriction that was independent of dose, indicating that all doses used saturated the number of functional α-adrenergic receptors. However, this method was sufficient to illustrate that the maximal vasoconstriction increased with developmental age, being greatest at 80% and 90%. In chickens, PE produced its greatest response in vascular rings from femoral and carotid arteries of 90% incubation embryos, suggesting there is a change in α-adrenergic receptor density (Le Noble et al., 2000). Therefore, while the maximal response pattern could have been related to a change in vascular density in alligator embryos, it also could have been due to changes in α-adrenergic receptor density of the vasculature.

Baroreflex features and mechanistic basis

The cardiac limb of the baroreflex was not functional at 60% of incubation and appeared for the first time at 70% of incubation age. This reflexive response was present when $\bar{P}$a was increased only. Reductions in $\bar{P}$a with SNP only triggered changes in heart rate in hatchlings and 1-year-old animals. The bradycardia that followed the hypertension was mediated by vagal activity as illustrated by the abolished response after atropine pre-treatment in 80% and 90% embryos and in hatchlings (Figs 8, 9). Interestingly, atropine itself had no effect on heart rate (J. Altimiras and D. A. Crossley, II, unpublished data), which is similar to what occurs in embryos of the white leghorn domestic chicken (Crossley and Altimiras, 2000). These results indicate that the vagus nerve is functional at least at 70% of incubation age but it is not tonically active. This pattern of a functional vagus without tone explains the presence of a hypertensive baroreflex, dependent on vagal inhibition, and the absence of a hypotensive baroreflex, which requires in part the withdrawal of vagal tone.

In addition to the withdrawal of vagal tone, sympathetic efferents constitute a secondary component of the hypotensive baroreflex in adult amniotic vertebrates (Altimiras et al., 1998; Korner, 1971). This mechanism may also be operational in hatchlings, as seen in animals pre-treated with atropine (Fig. 9). The slight but significant tachycardia recorded after the hypotensive bout with SNP (3 beats min^{-1}) is probably due to sympathetic activation, although the possible activation of other cardiac reflexes cannot be discarded without further experimentation. However, the late appearance of sympathetic innervation of the heart in other *in ovo* developing embryos (Higgins and Pappano, 1979) would indicate that this secondary mechanism is not involved in the embryonic alligator baroreflex.

The next prominent feature of the cardiac limb of the baroreflex during alligator development is its apparent rapid resetting at the time of the initial onset (70% and 80% of incubation time). While the mechanisms involved in baroreflex resetting cannot be resolved without further experimentation, it is relevant to discuss the different alternatives that could explain it. Rapid resetting was observed at 70% and 80% of incubation, as indicated by the initial pressure-induced bradycardia returning rapidly to control levels while arterial pressure continued to rise (Fig. 3). At 90% of incubation, the pattern was similar to that found in hatchlings and 1-year-old alligators, with a sustained bradycardia occurring until $\overline{P}$a returned to the control values (Fig. 3). Several mechanisms are involved in rapid or acute resetting of the baroreflex. The best-characterized mechanism relates to an alteration of vessel wall mechanics where baroreceptive endings are located (Eckberg, 1977). At the onset of a rapid increase in pressure, the tissue of the vessel wall lengthens due to viscoelastic properties. As a result, the baroreceptors are unloaded even when the overall vessel diameter or the strain on the vessel wall is increased (Chapleau et al., 1988). The acute resetting observed in the present study could therefore be related to developmental changes in the anatomic structure of the vessel wall where the baroreceptive endings are located. In reptiles and birds, the main baroreceptive area is the proximal truncus and the aortic arch (Backhouse et al., 1989; Berger, 1987; Smith, 1994). In this light, it is relevant that the development of the aortic wall in other *in ovo* developing embryos occurs in two distinct anatomic steps. In embryonic chickens, until 85% of incubation (day 18) the diameter of the aorta is increased by deposition of new cell layers (Arciniegas et al., 1989). Later in development the increased aortic diameter is due to an increased lumen of the vessel (Arciniegas et al., 1989). If aortic development occurs in alligators as in chickens, changes in the mechanical properties of the aortic wall during development may be linked to changes in baroreflex gain and acute resetting.

These structural changes may also account for the chronic resetting of the baroreceptors required as $\overline{P}$a increases during development (Fig. 2). Chronic resetting of the baroreflex allows this mechanism to remain operational within a physiological range of arterial pressures during vertebrate development (Segar, 1997). This process was evident by the continuous increase in the operational point of the baroreflex as alligator development progressed ($\overline{P}$a where baroreflex gain is maximal; referred to as $\overline{P}a_{G50}$ in Table 1). However, the exact mechanisms that underlie this process are unknown.

Maturation of baroreflex gain

Change in baroreflex gain is an important quantitative measure of baroreflex maturation during development. In this context, the experimental approach used in this study was limited because changes in peripheral resistance (the peripheral limb of the baroreflex) were not considered. However, given that sympathetic vascular regulation is absent in the fetal sheep until term (for a review, see Segar, 1997) and in the domestic chicken until the final 10% of incubation (Crossley et al., 2003; Mulder et al., 2002), quantification of the cardiac limb alone was considered a good approximation to the baroreflex. Absolute baroreflex gain was maximal at 80% development (41.22 beats kPa^{-1} min^{-1}), and dropped thereafter, reaching a minimum in 1-year-old alligators (9.69 beats kPa^{-1} min^{-1}; Table 1). It is possible, however, that absolute gain cannot be used as a measure of baroreflex regulation at different developmental ages. This is based on the fact that the observed changes in baroreflex gain are accompanied by important changes in resting cardiovascular parameters, as illustrated by the drop in heart rate seen in hatchling alligators (Fig. 1), which remains low in adult animals (Fig. 6). Thus, embryonic alligators have lower arterial pressure and higher heart rates than hatchlings and adults. If this is accounted for and baroreflex gain is calculated relative to resting arterial pressure and heart rate ($G_{50,norm}$; Table 1), a different picture appears. Among embryonic stages, maximal relative gain was still seen at 80%, but the normalized maximal gain increases first in hatchlings and then in 1-year-old alligators. This is due to the fact that smaller changes in gain have a greater influence on blood pressure regulation in systems operating at higher pressures and lower heart rates. This implies that baroreflex regulation is more important as alligator embryos develop from 80% to hatching.

In summary, baroreflex regulation appears during embryonic development with a substantial gain. This suggests that during embryonic development the baroreflex is necessary for proper cardiovascular maturation. Further differences in the embryonic developmental process between alligators and chickens may require the baroreflex to become functional earlier in embryonic alligators. Therefore, a delayed onset of baroreflex function is not a feature of embryonic development in an egg case.

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