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Maturation of cardiovascular control mechanisms in the embryonic emu (*Dromiceius novaehollandiae*)

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Summary

Our understanding of avian embryonic cardiovascular regulation has been based on studies in chickens. The present study was undertaken to determine if the patterns established in chickens are generally applicable to the emu, a ratite bird species. We studied cardiovascular physiology over the interval from 60% to 90% of the emu's 50-day incubation period. During this period, embryonic emus exhibit a slight fall in resting heart rate (from 171 beats min^{-1} to 154 beats min^{-1}) and a doubling of mean arterial pressure (from 1.2 kPa to 2.6 kPa). Exposures to 15% or 10% O_2 initially decreased heart rate during the first period of emu incubation studied [60% of incubation (60%I)] but increased heart rate in the 90%I group. Arterial pressure responded to hypoxia with an initial depression (–1.6 kPa) at 60%I and 70%I but showed no response during the later periods of incubation (80%I and 90%I). In addition, tonic stimulation of both cholinergic and adrenergic (α and β) receptors was present on heart rate at 70%I, with the cholinergic and β-adrenergic tone increasing in strength by 90%I. Arterial pressure was dependent on a constant β-adrenergic and constant α-adrenergic tone from 60%I to 90%I. A comparison with embryonic white leghorn chickens over a similar window of incubation revealed that emus and white leghorn chickens both possess an adrenergic tone on heart rate and pressure but that only emus possess a cholinergic tone on heart rate. Collectively, these data indicate that the maturation of cardiovascular control systems differs between white leghorn chickens and emus, inviting investigation of additional avian species to determine other patterns.

Key words: cardiovascular regulation, embryogenesis, physiology, incubation period, cholinergic tone, adrenergic tone, emu, *Dromiceius novaehollandiae.*

Introduction

Our understanding of cardiovascular regulation during avian development is primarily derived from embryonic chickens. However, the degree to which the developmental patterns in embryonic chickens may apply to other bird species is unknown. Given the diversity within this group of vertebrates, differences in physiological development among species might be anticipated. This point is supported by the differences in autonomic tone determined in two different strains of adult chickens (Kuo et al., 2001). However, Pearson and Tazawa (1999) recently compared heart rate changes during avian ontogeny and suggested that many altricial species may share similar patterns of cardiovascular maturation during early development. They documented the relationship of changes in heart rate with embryonic mass and incubation length of several altricial bird species. Given this relationship, the activation of the underlying mechanisms that regulate heart rate (as well as other cardiovascular parameters) may also occur at similar times during maturation among avian species.

Studies have established that chickens exhibit several components of the mature cardiovascular regulatory system early in ontogeny (Ignarro and Shideman, 1968; Pappano, 1977). These include responsive adrenergic and cholinergic receptor populations in the developing cardiovascular system as well as anabolic and catabolic enzymes for catecholamines and acetylcholine (Berry, 1950; Zacks, 1954; Ignarro and Shideman, 1968). Despite the presence of regulatory components from both arms of the autonomic nervous system, at day 12 [60% of the incubation period (60%I)] embryonic white leghorn chickens (*Gallus gallus*) possess only a tonic adrenergic stimulation of the cardiovascular system (Crossley and Altimiras, 2000). Several factors may dictate the timing of onset and subsequent effectiveness of cardiovascular regulatory mechanisms during chicken development. However, due to the lack of data from any avian species other than chickens, comparative hypotheses focusing on cardiovascular control during development cannot be evaluated.

Ratite birds are a useful group for addressing evolutionary hypotheses focusing on developmental cardiovascular physiology in birds. Ratites represent a distinct lineage of avian vertebrates, separated from other groups. Thus, the study of ratites could further the understanding of cardiovascular control patterns that are conserved during development in avian lineages (Burggren and Crossley, 2002).

The goal of this study was to characterize the development of cardiovascular control systems in the emu. We hypothesized that, given the taxonomic differences between emus and chickens, emus would possess different cardiovascular regulation mechanisms. Cardiovascular responses (change in heart rate and arterial pressure) to graded levels of hypoxia were used as indicators of the embryonic emus' ability to respond to the environment as well as to determine the timing of onset of a functional chemoreflex. Furthermore, cholinergic and adrenergic receptor antagonists were applied during the interval from 70%I to 90%I to assess the function of these receptors during this 30% developmental window of emu incubation.

Materials and methods

Animals and incubation

Freshly laid emu (*Dromiceius novaehollandiae*) eggs (*N*=20) were collected from adult holding pens at the Cross Timbers Emu Ranch (Flower Mound, TX, USA) and refrigerated at 3°C for a maximum of one week to temporarily arrest development pending collection of a full cohort of eggs. All eggs were then transported to the University of North Texas and placed in a force ventilation incubator (G.Q.F. Model 1536E) at 36°C with a relative humidity of 30%. Eggs were removed from incubation for study on days 30, 35, 40 and 45, which corresponded to 60%I, 70%I, 80%I and 90%I of a 50-day incubation period.

Surgical procedure

Eggs were placed in a water-jacketed temperature control dish for surgery. A 3 cm^2 window was cut in the shell to expose the vessels of the underlying chorioallantoic membrane (CAM). Both a CAM artery and vein were occlusively catheterized using heat-pulled polyethylene tubing (PE-90) filled with heparinized saline (0.9%) (Crossley and Altimiras, 2000).

Following catheterization, eggs were placed in a 1200 ml temperature-controlled chamber fitted with a lid containing multiple ports for externalization of catheters and inflow tubes for changing gas mixtures within the chamber. The arterial catheter was then passed through a port and connected to a pressure transducer (WPI) attached to a bridge amplifier (CB Sciences 400). Humidified 36°C air was continuously circulated into each experimental chamber during the period of study at a rate of 1.5 litres min^{-1}. This rate of gas flow was also used during the acute hypoxic response experiments. A data acquisitions system (Powerlab; AD Instruments, Colorado Springs, CO, USA) was used to collect the output signal at a sampling frequency of 100 Hz.

Experimental protocol

Eggs were equilibrated in their experimental chamber for 1 h to allow cardiovascular values to stabilize. Prior to experimental manipulations, baseline data were collected from 20 instrumented embryos for 30 min. Arterial pressure was recorded directly and heart rate determined from the blood pressure trace using a software tachograph. Two experimental series were conducted on each instrumented embryo.

Acute hypoxia response

20 embryos were used during this series. Each embryo was exposed to 15% O_2 for 5 min while changes in heart rate and arterial pressure were recorded. After a 30 min recovery period (a length of time that was sufficient for cardiovascular variables to return to control levels), the hypoxic procedure was then repeated with 10% O_2. This hypoxic protocol was completed in all of the embryos at each of the incubation intervals tested.

Cholinergic and adrenergic tone

Following a 1.5 h recovery period, five embryos from three stages (70%I, 80%I and 90%I; total *N*=15) from the hypoxic series study were used to assess the autonomic tone on the emu cardiovascular system. Three autonomic receptor antagonists were serially injected to determine the endogenous autonomic receptor (cholinergic and adrenergic) tone on heart rate and arterial pressure.

Following the hypoxic recovery period, a single dose (1 mg kg^{-1}) of the muscarinic antagonist atropine was injected to determine the resting cholinergic receptor stimulation (tone) on heart rate and arterial pressure. Once a new baseline for heart rate and pressure was established, a second drug, the β-adrenergic antagonist propranolol (3 mg kg^{-1}), was injected into each embryo. Heart rate and blood pressure changes were recorded and allowed to stabilize at a new resting level prior to the injection of the α-adrenergic antagonist phentolamine (3 mg kg^{-1}).

After the injection of phentolamine, blood samples were taken to determine catecholamine concentration in a subset of embryos. Blood samples (approximately 100 μl) were collected by allowing blood to flow freely from the arterial catheter into a 500 μl Eppendorf collection tube. Catecholamine blood samples were mixed with 5 μl of an EGTA/glutathione solution (0.2 mol l^{-1}/0.2 mol l^{-1}) to prevent catecholamine oxidation and immediately centrifuged at 13 700 ***g*** for 5 min. High-performance liquid chromatography (HPLC) analysis of plasma catecholamines was carried out as previously described by Fritsche and Nilsson (1990). Samples were maintained at –70°C until analysis was carried out (within one month). These embryos were then euthanized with an intravenous injection of sodium pentobarbital (50 mg kg^{-1}). All embryos were then weighed to the nearest 1.0 g to determine wet mass of the animals (Table 1).

Calculations and statistics

Non-parametric tests were conducted on the data due to small sample sizes and the comparison of percentage changes. A Wilcoxon non-parametric test was used to assess statistical significance of the responses to treatments (hypoxia and drug administration; atropine, propranolol and phentolamine) at each stage of incubation studied in the emu. A Mann–Whitney *U* test on the percentage change in heart rate (f_H) and mean arterial pressure ($\bar{P}a$) following each treatment was used to determine differences in responses between developmental intervals. Since repeated tests were carried out, thereby using the same data more than once, the fiduciary limit ($P<0.05$) was Bonferroni-corrected according to the number of times (2–3 times) each data set was used (thereby comparing 60%I to 70%I, 70%I to 80%I, 70%I to 90%I and 80%I to 90%I). This procedure was conducted to reduce the possibility of incorrectly finding a difference between incubation groups, i.e. a type 1 error. A Mann–Whitney *U* test was also conducted on the catecholamine levels using the same correction described above. The limited sample size prevented comparisons of the data acquired for the 60%I embryos. All data are shown as means ± 1 S.E.M. A fiduciary level of $P<0.05$ was used. Statistical analysis was conducted using Statistica (Statsoft, version 5.0).

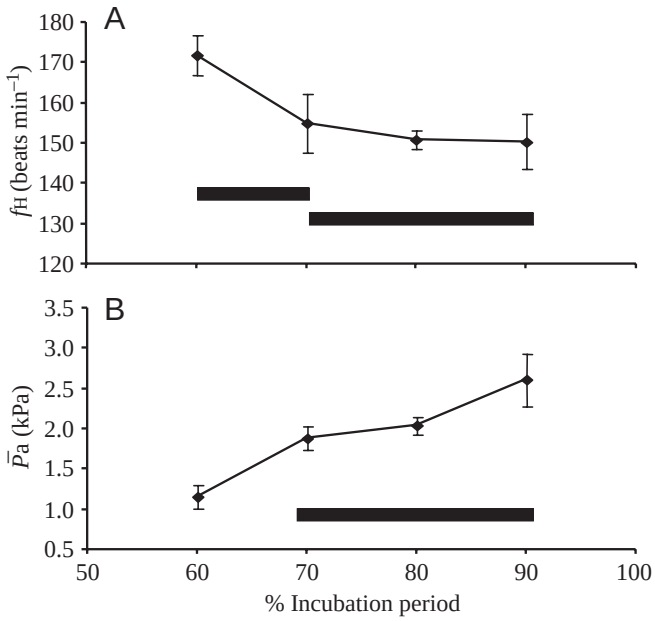

Fig. 1. Control developmental patterns for (A) heart rate (f_H) and (B) mean arterial pressure ($\bar{P}a$) in embryonic emus between 60% and 90% of incubation. A bar that overlaps different percentages of incubation indicates that the values are statistically similar. Data are presented as means ± 1 S.E.M. N=5 for each incubation period studied.

Results

Ontogenic cardiovascular changes

Resting f_H in embryonic emus decreased significantly from 171±5 beats min^{-1} at 60%I to 154±6 beats min^{-1} at 80%I and then remained constant during the rest of development (Fig. 1A). $\bar{P}a$ increased significantly from 1.2 kPa at 60%I to approximately 2.0 kPa at 70%I, showing no further significant increase during development (Fig. 1B).

Hypoxic responses

Hypoxic exposure (both 10% and 15% O_2) significantly decreased f_H at 60%I but had no effect from 70%I to 80%I. At 90%I, 10% O_2 caused a significant increase in f_H (Fig. 2A,C). The fall in f_H at 60%I was accompanied by a reduction in $\bar{P}a$ during exposure to 15% and 10% O_2 (Fig. 2B,D). A significant decrease in $\bar{P}a$ was also recorded during 10% O_2 in 70%I embryos and during 15% O_2 in 80%I embryos (Fig. 2B,D). However, hypoxia (10% and 15%) had no effect on $\bar{P}a$ in embryonic emus that had reached 90%I (Fig. 2B,D).

Autonomic blockade response

Fig. 3 represents the response of f_H and $\bar{P}a$ of an embryonic emu at 90%I to injections of cholinergic and adrenergic receptor antagonists. Following cholinergic blockade, f_H rose while $\bar{P}a$ was unchanged. β-adrenergic receptor blockade then caused a decrease in f_H and an increase in $\bar{P}a$. α-adrenergic receptor blockade caused both f_H and $\bar{P}a$ to fall (Fig 3A,B).

At all incubation ages tested, f_H significantly increased following cholinergic blockade: 70%I (increase of 12±3 beats min^{-1}), 80%I (increase of 20±3 beats min^{-1}) and with the greatest increase at 90%I (increase of

Table 1. *Emu egg mass, embryonic mass, volume of drug injection, saline flush, total volume injected and estimated blood volume of embryonic emus during the study period*

% Incubation	Egg mass (g)	Embryo wet mass (g)	*N*	Embryo blood volume (ml)	Drug volume (ml)	Saline volume (ml)	Total volume (ml)
60	580±11	44±2	5	3.05	0.60	0.12	0.51
70	560±8	108±3	5	7.56	0.10	0.20	0.90
80	559±9	172±5	5	12.07	0.10	0.20	0.90
90	544±10	222±12	5	15.56	0.10	0.20	0.90

Blood volume is calculated as 7% of the wet mass of the embryo. Egg mass data and embryonic wet mass of the emu are presented as means ± S.E.M. and number of embryos (*N*) used for each percentage of incubation studied.

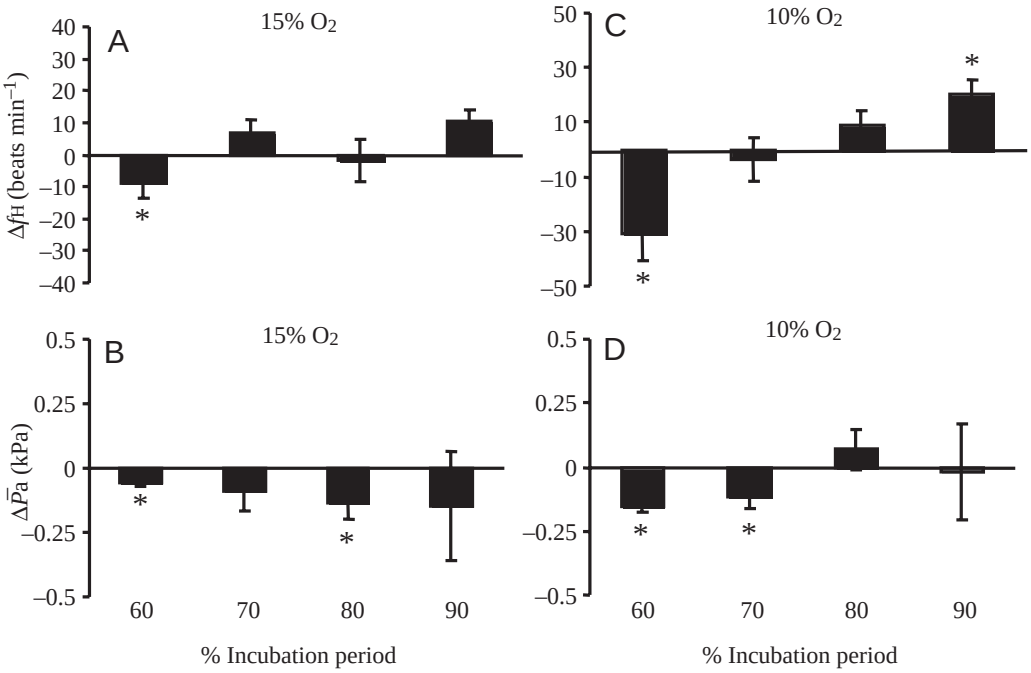

Fig. 2. Absolute changes in (A,C) heart rate (f_H) and (B,D) mean arterial pressure ($\bar{P}a$) in response to hypoxia (15% and 10% O_2) in embryonic emus. Asterisks indicate significant difference ($P<0.05$) from zero. All data are presented as means ± 1 S.E.M. $N=5$ for each incubation period studied.

50±3 beats min^{-1}) (Fig. 4A). Cholinergic blockade had no effect on $\bar{P}a$ during this period (Fig. 4B).

β-adrenergic blockade with propranolol, following cholinergic blockade, caused changes in f_H and $\bar{P}a$ at each measured age of emu development (Fig. 5A,B). Propranolol elicited a bradycardia that was significantly weaker at 70%I (–33 beats min^{-1}) compared with at 80%I (–43 beats min^{-1}) and 90%I (–46 beats min^{-1}) (Fig. 5A). Propranolol had the opposite effect on arterial pressure, which increased at 70%I (0.15 kPa), 80%I (0.3 kPa) and 90%I (0.9 kPa) (Fig. 5B). But although the absolute change in pressure caused by propranolol appeared greatest in the 90%I age group, the change in pressure relative to the resting arterial pressure for each age group was constant.

Injection of the non-specific α-adrenergic blocker phentolamine, after muscarinic and β-adrenergic blockade, produced a significant decrease in f_H and $\bar{P}a$ (Fig. 3A,B). Phentolamine decreased f_H by a similar amount in embryos at 70%I (–19 beats min^{-1}), 80%I (–28 beats min^{-1}) and 90%I (–25 beats min^{-1}) (Fig. 5C). Phentolamine injection also caused a significant decrease in $\bar{P}a$ at 70%I (–0.35 kPa), 80%I (–0.60 kPa) and 90%I (–1.50 kPa) (Fig. 5D). Again, while the absolute change in pressure caused by phentolamine appeared greatest in the 90%I age group, the change in pressure relative to the resting arterial pressure for each age group was constant.

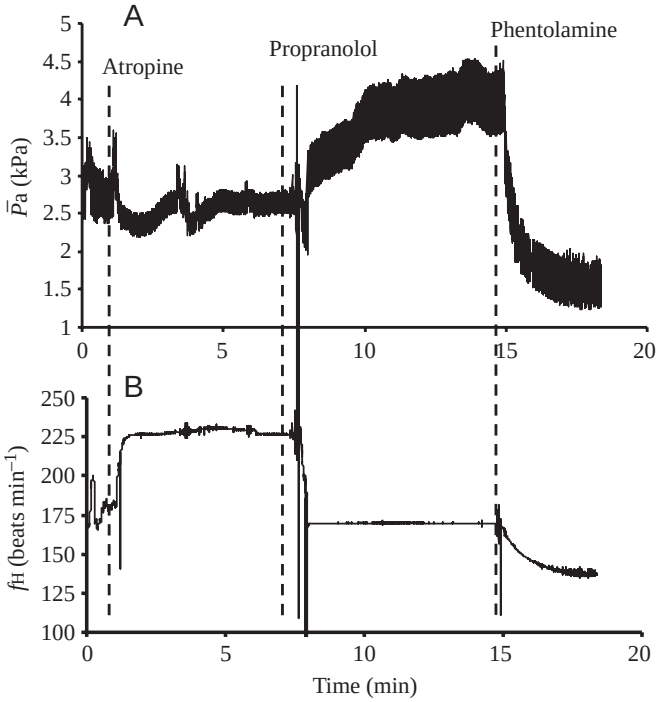

Fig. 3. A representative trace from a single embryonic emu (90%I) illustrating the (A) arterial pressure ($\bar{P}a$) and (B) heart rate (f_H) responses to injections of the autonomic blockers atropine, propranolol and phentolamine. The broken vertical lines indicate times of drug injections.

Changes in circulating catecholamines

Plasma concentrations of norepinephrine increased progressively with embryonic development to a maximum of 80.0±21.0 ng ml^{-1} at 90%I (Table 2). The maximum epinephrine level was 53±24 ng ml^{-1} at 90%I, but the levels did not differ statistically during incubation (Table 2).

Discussion

This study was conducted to understand the development of cardiovascular regulation in embryonic emus and provide additional information about developmental cardiovascular

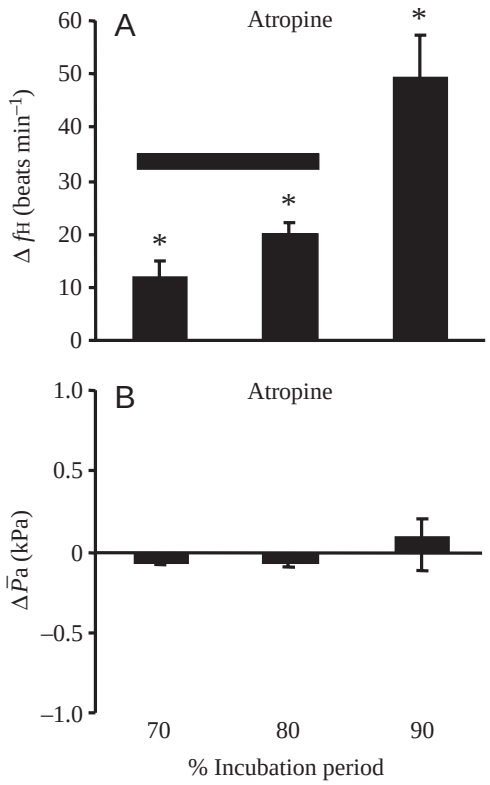

Fig. 4. Absolute changes in (A) heart rate (f_H) and (B) mean arterial pressure ($\bar{P}a$) in response to atropine. Asterisks represent significant (P<0.05) drug responses, and a bar that overlaps different percentages of incubation indicates that the values are statistically similar between these periods. Data are presented as means ± 1 S.E.M. N=5 for each incubation period studied.

Table 2. *Mean plasma norepinephrine and epinephrine levels during development*

% Incubation	Norepinephrine (ng ml^{-1})	Epinephrine (ng ml^{-1})
60	15±6 (3)	14±7 (3)
70	27±6 (5)	13±8 (5)
80	64±17 (5)*	21±11 (5)
90	80±21 (5)*	53±24 (5)

An asterisk indicates significant differences (P<0.05) from adjacent periods without asterisks. Due to limited sample size, statistics on catecholamines were omitted for 60% of incubation. All measures are presented as means ± S.E.M. for each percentage of incubation studied. The number of embryos is presented in parentheses.

physiology in birds. Basal cardiovascular function comes under tonic adrenergic and cholinergic control between 60%I and 70%I. The cardiovascular responses to hypoxia during embryonic development suggest that reflexive control systems may be operational in emus prior to hatching. When compared with the known cardiovascular regulation systems in embryonic white leghorn chickens, emu embryos possess some similar cardiovascular control mechanisms but there are important differences between these species during development.

Critique of the experimental protocol

The heart rate and arterial pressure responses to hypoxia and reaction to autonomic antagonists were conducted in all

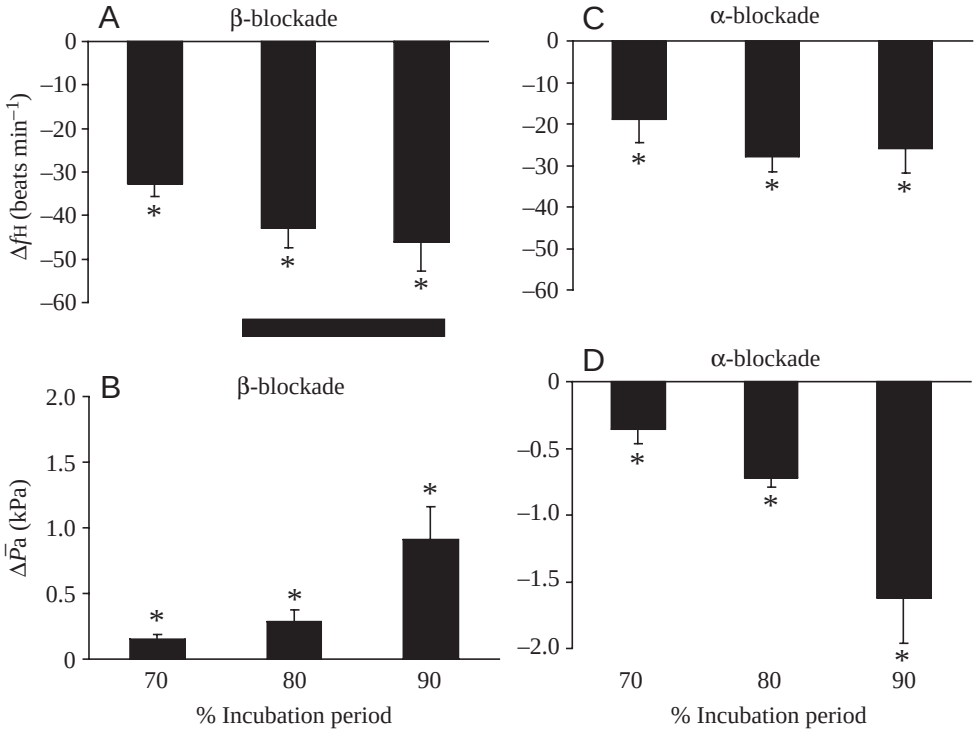

Fig. 5. Absolute changes in (A,C) heart rate (f_H) and (B,D) mean arterial pressure ($\bar{P}a$) in response to propranolol (A,B) and phentolamine (C,D). Asterisks represent significant (P<0.05) drug responses, and a bar that overlaps different percentages of incubation indicates that the values are statistically similar between these periods. Data are presented as means ± 1 S.E.M. N=5 for each incubation period studied.

embryos tested at 70%I, 80%I and 90%I. A recovery period between the hypoxic exposures and autonomic blockade allowed blood chemistry [lactate, pH, oxygen partial pressure (P_{O_2}) and carbon dioxide partial pressure (P_{CO_2})] changes associated with hypoxia to return to control values. The recovery period (1.5 h) was based on blood chemistry recovery profiles following 5 min hypoxic exposures (15% and 10% O_2) in embryonic white leghorn chickens. Following each hypoxic exposure in embryonic white leghorn chickens, blood parameters returned to near control values within 1 h (D. A. Crossley, unpublished data). Therefore, the time period allowed prior to the initiation of the autonomic blockade series (1.5 h) in embryonic emus was probably sufficient to allow blood chemistry to return to control levels.

The validity of utilizing a 5 min exposure to assess the cardiovascular response of embryonic emus also must be addressed from two views. The first is that the 5 min period would not allow the P_{O_2} to reach the desired level within the experimental chamber. The second is that the exposure period was sufficient to elicit a transient cardiovascular response only. While both of these issues are acknowledged, the flow rate of air into the chambers was sufficient to turn over the volume of gas to the new P_{O_2} level in approximately 30 s. This was independently verified prior to each study (D. A. Crossley et al., unpublished data). Therefore, the chamber that the embryo was in reached the desired P_{O_2} within the first minute. A 5 min exposure could also be questioned as insufficient in terms of allowing an equilibrium cardiovascular state to be reached by the embryo. The goal of these experiments was to determine the acute cardiovascular response to hypoxia in embryonic emus in an effort to study the cardiovascular chemoreflex and compare it with the response of embryonic white leghorn chickens. The changes in the acute hypoxic response (Fig. 2) and differences from that of the white leghorn chicken embryos indicates that a 5 min period was sufficient to assess the developmental and species response differences to acute hypoxia.

Each of the autonomic blockade agents in the study was given to each embryo in series (atropine, propranolol and then phentolamine). This method of study has been used successfully in embryonic chickens (Girard, 1973; Tazawa et al., 1992; Crossley and Altimiras, 2000), embryonic alligators (D. A. Crossley et al., unpublished data) and larval bullfrogs (Burggren and Doyle, 1986) to determine the presence and strength of cardiovascular autonomic tone during ontogeny. Therefore, this method was chosen to allow comparison with existing literature for other species. However, there is a potential for the cardiovascular responses to differ based on the order of drug injection, and this should be considered in all studies.

Drug injection volumes might have induced a hypervolemia in the developing emu. Blood volumes in embryonic emus (Table 1) were estimated using the known relationship between embryonic mass and blood volume in developing chickens (Crossley and Altimiras, 2000). Each drug injection and flush volume totaled maximally less than 6% of the estimated blood volume in the earliest embryos (60%) studied. Estimated blood volume of the developing emu was calculated as 7% of the embryonic wet mass (Table 1) based on previously determined values for embryonic emus (D. A. Crossley, unpublished data). Therefore, the maximal volume of fluid added to the embryo during the length of the study was less than 17% of the estimated blood volume. Using 7% of embryonic mass as an estimation of blood volume may underestimate the actual volume by as much as 25% (based on chickens; Romanoff, 1967). Therefore, the volume of each injection probably did not alter cardiovascular function (Crossley and Altimiras, 2000).

Developmental changes: pressure and heart rate

Over the interval of emu development tested (60%I to 90%I), resting $\bar{P}$a increased progressively and was significant only between 60%I and 70%I (Fig. 1B). Routine f_H gradually decreased over this time frame, possibly due to the onset and progressive expression of parasympathetic cardiac regulation (Fig. 1A). This speculation is based on the observation that atropine produced progressively larger increases in f_H after 60%I (Fig. 4A). If this explanation is correct, ongoing maturation of the cholinergic inhibitory system seems likely to continue after hatching because f_H was elevated (3.5 times higher) compared with adult emus (Grubb et al., 1983). While this finding could be attributed to mass changes alone, maturation of cholinergic receptor-mediated regulation may also contribute.

Cardiovascular response to hypoxia

60%I embryonic emus responded to acute hypoxia (15% or 10% O_2) with a bradycardia, while 90%I embryos exhibited a tachycardia during 10% O_2 exposure (Fig. 2). This reversal suggests that at 90%I embryonic emus may possess central or reflexive regulation of f_H. The sensory components that detect changes in Pa_{O_2} may be functional and the effector systems altering f_H are active. If the components of the f_H chemoreflex are intact at 90%I, as the data indicate, then the hypoxic tachycardia was probably induced by a reduction of cholinergic depression and/or an increase in adrenergic stimulation of f_H. The latter has been demonstrated in other avian species and could be largely responsible for the response documented in this study (Giussani et al., 1994; Dragon et al., 1996; Mulder et al., 2000; Crossley et al., 2003).

The change in the $\bar{P}$a response to hypoxia over development suggests that a hypoxic reflexive becomes operational during emu incubation. This suggestion is based on the pressure response change from hypotension during 10% O_2 (60%I and 70%I) to no change in pressure in embryos at 80%I and 90%I (Fig. 2B,D). An alternative explanation for no change in $\bar{P}$a in the 80%I and 90%I groups may be a reduced sensitivity to hypoxia. However, since 10% O_2 was sufficient to induce changes in f_H at 90%I, it is likely that the embryos were sensitive to hypoxia at this point (Fig. 2B).

Regulation of heart rate and blood pressure

Adrenergic tone on heart rate

In addition to a cholinergic tone, embryonic emus also had an important β-adrenergic receptor-mediated tone on f_H

(Fig. 5A). This β-adrenergic tone rose between 70%I and 80%I (Fig. 5A). In chicken embryos, there is an increase in β-adrenergic tone on f_H that has been partially attributed to an increased stimulation by elevated levels of circulating catecholamines (Dragon et al., 1996; Crossley and Altimiras, 2000; Mulder et al., 2000). Circulating levels of norepinephrine also increased from 70%I to 80%I (Table 2) and may account for the increase in β-adrenergic receptor-mediated tone on f_H during this period.

Unlike the f_H responses to β-blockade during emu development, the reaction to α-blockade with phentolamine was constant during the period of incubation studied (70%I to 90%I; Fig. 5C). The change in f_H after α-blockade may be derived from a secondary response to the accompanied vasodilation, which would increase filling time and reduce f_H. However, given the excitatory action of cardiac α-receptors in adult, as well as neonatal and fetal, mammals (Cohen, 1986), direct α-receptor excitatory tone on f_H may be present in embryonic emus.

Adrenergic tone on pressure

Embryonic emus possessed a constant β-adrenergic tone that decreased $\bar{P}a$ during incubation when corrected for the developmental changes in resting arterial pressure (Fig. 5B). These embryos also possessed a constant α-adrenergic tone that increased $\bar{P}a$ (Fig. 5D). In combination, these findings indicate that the embryonic emu relies on both α- and β-adrenergic receptor types to maintain $\bar{P}a$ during the window of development studied.

Comparison with white leghorn chickens

The development of cardiovascular regulation in birds has been studied primarily in the domestic chicken. The present study reveals some distinct differences between emus and chickens.

In emus, f_H initially falls (between 60%I and 70%I) then remains relatively constant (Fig. 1A), while embryonic chickens maintain f_H over the same period of incubation (Girard, 1973; Tazawa, 1981). This difference may be attributed to the presence of a cholinergic tone on f_H in emus (Fig. 4A) and its absence in chickens of the white leghorn strain (Crossley and Altimiras, 2000). Furthermore, the hypoxic tachycardia of late embryonic emus (90%I; Fig. 2C) differs from the bradycardia in white leghorn embryos. Again, this may also be attributed to the differences in cholinergic tone between the species.

Differences in the mechanisms of regulation were also evident between embryonic emus and chickens of the white leghorn strain. The most prominent difference between these avian embryos was the presence of cholinergic tone on f_H in embryonic emus (present study) and the absence in white leghorn chickens (Crossley and Altimiras, 2000). Given our understanding of these two models of avian development, the reason for the appearance of cholinergic f_H tone in emus and its absence in white leghorn chickens is unclear. This comparison illustrates that the cardiovascular regulatory mechanisms delineated in white leghorn chickens during development may be different in other bird species.

Conclusions

During embryonic development, emu cardiovascular regulation mechanisms are more advanced that those of embryonic white leghorn chickens over a similar window of development. At 70%I, a clear cholinergic receptor-mediated tone (regulating f_H) and adrenergic receptor-mediated tone (regulating f_H and $\bar{P}a$) are functional in the emu. As the emu matures, the embryo relies on an increasing cholinergic tone on f_H and a β-adrenergic control of f_H. Furthermore, embryonic emus exhibit temporal changes in the cardiovascular responses to 10% O_2. Therefore, maturation of cardiovascular regulatory mechanisms should be expected to differ between avian species during development.

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