Ontogeny of cholinergic and adrenergic cardiovascular regulation in the domestic chicken (Gallus gallus)

DANE CROSSLEY II and JORDI ALTIMIRAS

1Department of Biological Sciences, University of North Texas, Denton, Texas 76201; and 2Danish Center for Respiratory Adaptation, Department of Zoophysiology, University of Aarhus, Aarhus 8000, Denmark

Received 27 December 1999; accepted in final form 8 May 2000

Crossley II, Dane, and Jordi Altimiras. Ontogeny of cholinergic and adrenergic cardiovascular regulation in the domestic chicken (Gallus gallus). Am J Physiol Regulatory Integrative Comp Physiol 279: R1091–R1098, 2000.—Adrenergic and cholinergic tone on the cardiovascular system of embryonic chickens was determined during days 12, 15, 19, 20, and 21 of development. Administration of the muscarinic antagonist atropine (1 mg/kg) resulted in no significant change in heart rate or arterial pressure at any developmental age. In addition, the general cardiovascular depressive effects of hypoxia were unaltered by pretreatment with atropine. In addition, the ganglionic blocking agent hexamethonium (25 mg/kg) did not induce changes in heart rate. The β-adrenergic antagonist propranolol (3 mg/kg) induced a bradycardia of similar magnitude on all days studied, with a transient hypertensive action on days 19–20, indicating the existence of an important cardiac and vascular β-adrenergic tone. Injections of the α-adrenergic antagonists prazosin or phentolamine (1 mg/kg) reduced arterial pressure significantly on all days of incubation studied. Collectively, the data indicate that embryonic chickens rely primarily on adrenergic control of cardiovascular function, with no contribution from the parasympathetic nervous system.

vagal tone; adrenergic tone; cardiovascular regulation

EMBRYONIC CHICKENS have long been used for the study and understanding of cardiovascular function as well as regulation during vertebrate ontogeny. Several studies have focused on the onset of neurohumoral cardiac regulation, but little is known of its functional significance during embryonic development. In addition, neurohumoral control of peripheral vascular beds, which is essential for blood pressure regulation, has been only poorly characterized.

Despite this limited knowledge, several morphological and physiological traits have been identified in developing chickens. Cardiac muscarinic and adrenergic receptors are present in embryonic chickens during the first quarter of incubation (3). In addition, the anabolic and catabolic enzymes of the primary autonomic neurotransmitters (acetylcholine and norepinephrine) are also present during early development (14, 34). Prior research using field stimulation methods established that autonomic efferents are capable of releasing acetylcholine from cholinergic fibers on day 12, whereas sympathetic release of norepinephrine is possible on day 21 of incubation (23). These data demonstrate the functional integrity of autonomic efferent pathways; however, they do not confirm the presence of a tonic cardiovascular regulation in embryonic chickens.

In embryonic chickens a functional cholinergic tone on the cardiovascular system would require the maturation of both afferent and central elements, which may not occur until later in development. Similarly, a functional adrenergic tone would require maturation of afferent and central elements of the sympathetic system; however, additional adrenergic tone may originate from blood-borne catecholamines. Circumstantial evidence suggests that a tonic regulation occurs in embryonic chickens (30). However, to our knowledge, a systematic study encompassing different developmental days with an appropriate statistical treatment of the data has not been completed.

Thus the present study was undertaken to further our understanding of neurohumoral cardiovascular regulation in developing avian embryos. Antagonists of the common receptors involved in cardiovascular regulation were administered throughout ontogeny (i.e., muscarinic and α- and β-adrenergic receptor antagonists) to address this issue. In addition, experiments were conducted with ganglionic blocking agents as well as hypoxic stress to further address the role of vagal function in cardiovascular control. Finally, given the potential for catecholamines originating from nonneural sources to target adrenoreceptors, a simultaneous determination of circulating plasma levels of catecholamines was conducted.

MATERIALS AND METHODS

Experimental animals. Experiments were carried out in two series conducted over the period from November 1998 to March 1999. Series I was conducted on chicken eggs of the Plymouth Russ208 strain purchased from Fællesrugeriet (Randers, Denmark) at the University of Aarhus. Series II
was conducted on chicken eggs of the White Leghorn strain purchased from the Texas A&M University poultry farm at the University of North Texas. On arrival eggs were placed in incubation at 38°C and 60–70% relative humidity and were turned automatically every 3 h. Embryos incubated for 12, 15, 19, 20, and 21 days of a 21-day incubation period were studied. The 20-day-old embryos were defined as internally pipped embryos, as verified by candling, and 21-day-old embryos were defined as externally pipped embryos. The work in Denmark was carried out under University of Aarhus permit number 1997-101-112. The work completed in the US was carried out under University of North Texas animal protocol UNT-00-01.

**Measurement of blood pressure and heart rate.** At selected developmental ages, eggs were removed from the incubator, candled to trace the major chorioallantoic arteries with a soft pencil, and placed in a vermiculite bath (38°C). A chorioallantoic membrane (CAM) artery was exposed by removal of a small portion of eggshell. The smallest branching vessel was cannulated to trace the major chorioallantoic arteries with a soft pencil, and placed in a vermiculite bath (38°C). A chorioallantoic membrane (CAM) artery was exposed by removal of a small portion of eggshell. The smallest branching vessel was occlusively cannulated with a polyethylene catheter (PE-90, Clay-Adams) with the tip heat-pulled to an outer diameter of ~0.5 mm under a dissection microscope. Silk suture was used to secure the catheter to the vessel after careful alignment of the two, and the catheter was glued to the eggshell with cyanoacrylic glue (VetBond 3M). On completion of the surgical procedures, each egg was returned to the experimental chamber. In **series I**, the experimental chamber consisted of a 500-ml stainless steel water-jacketed trough fitted with a lid containing four circular openings. Eggs were placed blunt end up on a small amount of vermiculite within the openings, which resulted in the egg resting within the trough below the level of the lid. Each opening was then covered with a 50-ml dome containing a 1-cm opening to allow passage of the arterial catheter as well as passive circulation of room air. In **series II**, the experimental chamber was modified to allow a precise control of the gas environment of the embryos. The glass water-jacketed chamber (300 ml) was fitted with a glass lid with three holes allowing the flushing of different gas mixtures as well as the connection of the catheter to blood pressure transducers. With this system each chamber was continually flushed with water-saturated room air that had been circulated through a heated sand bath at 37°C. Oxygen content (% O2) in the chamber was continuously monitored with an oxygen analyzer (model S 3A-1, Applied Instruments).

Blood pressure traces were obtained via fluid-filled catheters connected to Statham pressure transducers (P23) that were calibrated against a static water column. The transducer was connected to a Beckman recorder (R511A) for proper amplification of the signal before being sampled at 500 Hz by a computer via a Data Translation card (DT2801A) and LabView custom-made acquisition software. Heart rate (fH) was obtained from the pressure signal. Zero-pressure reference for the system was completed as previously described (1). Briefly, a nominal “zero point” was set at the top edge of the eggshell for the entire study. Once the experiment was completed, the embryo was euthanized and quickly frozen at −20°C. After this procedure, the egg was cut along the longitudinal axis to determine the relative position of the embryo and the heart in relation to the top of the eggshell. An offset correction of all pressure data was then conducted to determine pressure on each day of study.

**Experimental protocol.** In each series the experiment included a sequential arterial injection of antagonists of the main receptors involved in adult vertebrate cardiovascular control. These blocking agents included atropine (1 mg/kg, Sigma) to characterize vagal tone, propranolol (3 mg/kg, Sigma) to characterize β-adrenergic tone, and prazosin or phentolamine (1 mg/kg for both drugs, Sigma) to characterize the role of α1- and α2-adrenergic receptors on the developing cardiovascular system. The efficacy of the antagonists was tested in preliminary experiments, because the antagonist blocked the effects of a subsequent injection of the agonist (Fig. 1). Injection volumes were normalized for each embryonic age to 5% of the total blood volume based on literature data (Table 1; Ref. 25). Each dose was calculated taking into account the total mass of living tissue in the egg (including total embryonic wet mass as well as the total mass of the egg membranes; Ref. 25). Special care was taken to avoid large volumes of dead space in injection lines with each drug administration.

Each injection was preceded by a 10-min recording period of preinjection values. After the injection of a drug, blood pressure and fH were recorded until they reached stable values (Table 1). The dose of each antagonist was titrated until a 10% depression of blood pressure was obtained. These blocking agents included atropine (1 mg/kg, Sigma) to characterize vagal tone, propranolol (3 mg/kg, Sigma) to characterize β-adrenergic tone, and prazosin or phentolamine (1 mg/kg for both drugs, Sigma) to characterize the role of α1- and α2-adrenergic receptors on the developing cardiovascular system. The efficacy of the antagonists was tested in preliminary experiments, because the antagonist blocked the effects of a subsequent injection of the agonist (Fig. 1).

**Table 1. Injection volumes and amount of antagonist drugs used on days of development**

<table>
<thead>
<tr>
<th>Day of Development</th>
<th>12</th>
<th>15</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vblood, ml</td>
<td>1.61</td>
<td>2.35</td>
<td>2.90</td>
<td>2.81</td>
<td>2.61</td>
</tr>
<tr>
<td>WMembryo, g</td>
<td>5.0</td>
<td>12.5</td>
<td>22.1</td>
<td>25.8</td>
<td>28.4</td>
</tr>
<tr>
<td>WMmembranes, g</td>
<td>3.9</td>
<td>5.2</td>
<td>4.9</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>V inj, total, µl</td>
<td>80</td>
<td>120</td>
<td>150</td>
<td>150</td>
<td>135</td>
</tr>
<tr>
<td>Atropine, µg</td>
<td>8.9</td>
<td>17.7</td>
<td>27.0</td>
<td>30.4</td>
<td>32.9</td>
</tr>
<tr>
<td>Prazosin, µg</td>
<td>26.7</td>
<td>53.2</td>
<td>80.9</td>
<td>91.3</td>
<td>98.7</td>
</tr>
<tr>
<td>Prazosin, µg</td>
<td>8.9</td>
<td>17.7</td>
<td>27.0</td>
<td>30.4</td>
<td>32.9</td>
</tr>
<tr>
<td>Phentolamine, µg</td>
<td>8.9</td>
<td>17.7</td>
<td>27.0</td>
<td>30.4</td>
<td>32.9</td>
</tr>
</tbody>
</table>

Vblood, estimated volume of blood; WMembryo, wet mass of the embryo; WMmembranes, wet mass of extraembryonic membranes including chorioallantoic and yolk membranes; V inj, total volume injected (= 1/3 drug + 2/3 flush volume); atropine, dose to equal 1 mg/kg; propranolol, dose to equal 3 mg/kg; prazosin, dose to equal 1 mg/kg; phentolamine, dose to equal 1 mg/kg. Values for Vblood, WMembryo, and WMmembranes are from Ref. 25.
values. In series II, oxygen concentration was measured and maintained at 20.9 ± 0.2% O₂.

In series I, a blood sample was obtained from five embryos on each incubation day studied, after the completion of the experimental protocol. Blood was allowed to flow freely from the arterial catheter because suction caused occlusion of the CAM vasculature. Blood samples were mixed with 5 µl of an EGTA-glutathione solution (0.2 M-0.2 M) to prevent catecholamine oxidation and immediately spun down to separate the plasma. Samples were maintained at −70°C until analysis was carried out (within 1 mo). HPLC analysis of plasma catecholamines was carried out as previously described (9).

Two additional experiments were carried out as part of series II in an attempt to further assess the tonic role of the vagus nerve in cardiac control. The first experiment involved an exposure to 10% O₂ for 5 min, followed by an injection of atropine (1 mg/kg), stabilization for 20 min, and reexposure to 10% O₂. Hypoxic gas concentrations were achieved by volumetric mixing of nitrogen and room air.

The second experiment consisted of the injection of hexamethonium chloride (25 mg/kg, Sigma), a ganglionic blocking agent. This dosage was within the range previously used for assessment of ganglionic activity in fetal and newborn mammals (1–25 mg/kg; Refs. 6, 15, 22, 27). In each experiment, six embryos at each incubation age were used. Total protocol length in each series was 1 h.

Statistics. Mann-Whitney U and Wilcoxon nonparametric tests were used to assess statistical differences between chicken strains (series I and series II), developmental days, and treatments (hypoxia and drug administration: atropine, propranolol, α-blocker, and hexamethonium). Because 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 three or four times because the tests between developmental days were restricted to adjacent days (thereby comparing days 12–15, 15–19, 19–20, and 20–21). All data are presented as means ± SE.

RESULTS

Comparison of control values between experimental series. The values obtained are in general agreement with prior research over this period of chicken development (13, 17, 32). Mean arterial pressure (MAP) rose in a similar manner (from 0.37 to 3.5 kPa) in each series from day 12 to day 21 of incubation (Fig. 2), with significant differences between series on day 20 only. In addition, ḟH on each day of incubation in each series showed no statistical difference (Fig. 2); therefore, the data from both series were pooled for further analysis.

Effects of cholinergic antagonists and hypoxia. The muscarinic antagonist atropine did not significantly alter MAP or ḟH on any developmental day studied, and the ganglionic blocker hexamethonium did not significantly alter MAP or ḟH in series II (Table 2). Similarly, the pronounced cardiovascular changes caused by hypoxia were unaltered by atropine treatment (Table 3); Fig. 3 presents the cardiovascular response to hypoxia without atropine treatment. All these findings are summarized in Table 3, which illustrates the predicted (as expected in adult chickens) vs. observed responses to each manipulation used to induce a vagal response.

Table 2. Heart rate and mean arterial pressure before and after injection of atropine and hexamethonium

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MAP</th>
<th>ḟH</th>
<th></th>
<th>n</th>
<th>MAP</th>
<th>ḟH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 12</td>
<td></td>
<td></td>
<td></td>
<td>Day 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>11</td>
<td>0.8 ± 0.9</td>
<td>199 ± 7</td>
<td>Post</td>
<td>11</td>
<td>0.8 ± 0.1</td>
<td>197 ± 8</td>
</tr>
<tr>
<td>Day 15</td>
<td></td>
<td>1.7 ± 0.1</td>
<td>212 ± 6</td>
<td></td>
<td></td>
<td>1.6 ± 0.1</td>
<td>210 ± 7</td>
</tr>
<tr>
<td>Day 19</td>
<td>13</td>
<td>2.5 ± 0.2</td>
<td>213 ± 7</td>
<td></td>
<td></td>
<td>2.6 ± 0.2</td>
<td>215 ± 8</td>
</tr>
<tr>
<td>Day 20</td>
<td>14</td>
<td>3.1 ± 0.2</td>
<td>237 ± 6</td>
<td></td>
<td></td>
<td>3.0 ± 0.2</td>
<td>239 ± 5</td>
</tr>
<tr>
<td>Day 21</td>
<td>14</td>
<td>3.5 ± 0.2</td>
<td>252 ± 5</td>
<td></td>
<td></td>
<td>3.5 ± 0.2</td>
<td>255 ± 7</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n, no. of animals tested on each day of incubation. MAP, mean arterial pressure; ḟH, heart rate; Pre, before injection; Post, after injection.
Effects of adrenergic antagonists. Propranolol injection caused a pronounced bradycardia (−38 ± 3 beats/min) on all days studied, with a transient pressure effect on days 19 and 20 (−0.33 ± 0.04 kPa) of incubation (Fig. 4, A and B). This significant $f_{IH}$ response to $\beta$-blockade changed in intensity with embryonic development (day 12 < day 15 = day 19 < day 20), as illustrated in Fig. 4B. Although developmental changes in response intensity have not been tested previously, all other cardiovascular responses are in agreement with those of a prior study on embryonic chickens aged 12 to 21 days (31).

The $\alpha$-adrenoceptor antagonists from each study series induced similar cardiovascular responses despite

### Table 3. Predicted and observed responses of MAP and $f_{IH}$ after atropine injection, atropine combined with hypoxia, and hexamethonium injection

<table>
<thead>
<tr>
<th>Day</th>
<th>Atropine Predicted</th>
<th>Atropine Observed</th>
<th>Atropine + 10% O$_2$ Predicted</th>
<th>Atropine + 10% O$_2$ Observed</th>
<th>Hexamethonium Predicted</th>
<th>Hexamethonium Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
</tr>
<tr>
<td>15</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
</tr>
<tr>
<td>19</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
</tr>
<tr>
<td>20</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
</tr>
<tr>
<td>21</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
<td>MAP, $f_{IH}$</td>
</tr>
</tbody>
</table>

Predicted responses are based on the response of adult animals. ↑, Increase; ↓, decrease; absence of an arrow indicates that no changes were observed.

### Fig. 3. Absolute change (Δ) in MAP (A) and $f_{IH}$ (B) induced by a 5-min exposure to 10% O$_2$ on each day of chicken incubation studied. An asterisk indicates a significant response to hypoxia ($P$ value 0.0167). Like letters represent similar responses between days. Data are plotted as mean absolute change ± SE; $n$ = 6 embryos for each day.

### Fig. 4. Absolute change in embryonic MAP (A) and $f_{IH}$ (B) caused by the injection of 3 mg/kg propranolol. An asterisk indicates significant response to drug injection ($P$ value 0.0125). Like letters represent similar responses between days. Data are plotted as mean absolute change ± SE; $n$ = 6 embryos for each day.
their differing specificity for \(\alpha_1\)- and \(\alpha_2\)-receptors (Table 4). Given this result, statistical analysis was conducted on the pooled data from each series. In general, \(\alpha\)-blockade resulted in a clear hypotensive bradycardia on all incubation days tested, with the notable exception of day 21 (Fig. 5, A and B). The extent of this response varied throughout development, with pressure changes ranging from \(-0.15 \pm 0.02\) kPa to \(-1.27 \pm 0.29\) kPa (Fig. 5A) and a bradycardia ranging from \(-12 \pm 2\) to \(-55 \pm 12\) beats/min (Fig. 5B).

**Plasma levels of catecholamines during development.**

The concentrations of norepinephrine, epinephrine, and dopamine showed significant changes as embryonic maturation proceeded (Table 5). Norepinephrine levels peaked on day 19 at 169.4 \pm 52.0 ng/ml, a level significantly higher than that on earlier days \((P < 0.05)\). Epinephrine exhibited a similar increase to 80.6 \pm 23.2 ng/ml on day 19, again significantly higher than that on earlier days \((P < 0.05)\). Finally, dopamine peaked at 9.1 \pm 1.3 ng/ml on day 20, significantly higher than that on earlier days \((P < 0.05)\).

**DISCUSSION**

Tonic regulation of cardiovascular function during late development in embryonic chickens exhibits distinct attributes that differ from those present in hatching as well as adult animals (28). This study has demonstrated that in embryonic chickens basal cardiovascular function is maintained primarily via tonic adrenoreceptor stimulation, whereas vagal tone remains absent.

**Cardiac regulation: vagal and \(\beta\)-adrenergic tones.**

Field stimulation of embryonic atrial tissue has previously shown that the release of acetylcholine from postganglionic synapses can occur as early as day 12 of incubation in chickens (23). Thus day 12 has traditionally been thought to represent the onset of parasympathetic activity in the heart, and, by extension, of the cardiovascular system in embryonic chickens.

The results presented in this study strongly suggest that, despite the completeness of a cholinergic effector pathway by day 12, there is no evidence for a tonic contribution to cardiac regulation during embryonic incubation. Previous studies have reported either that atropine lacks chronotropic actions in chicken embryos from day 13 to day 16 of incubation (30) or that it produces a tachycardia in a 17-day-old embryo (13). Both studies lacked an appropriate statistical treatment of the data, thereby limiting the conclusions that could be drawn. It is acknowledged that our findings appear to conflict with those of a previous study (13) attempting to investigate the ontogeny of vagal function in embryonic chickens. It is important to recognize, however, that the findings in this study cannot be

---

**Table 4. Effect of prazosin (series I) and phentolamine (series II) on different days of development**

<table>
<thead>
<tr>
<th>Day</th>
<th>(\Delta MAP, \text{kPa} )</th>
<th>(\Delta f_H, \text{beat/min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prazosin</td>
<td>Phentolamine</td>
</tr>
<tr>
<td>12</td>
<td>0.15 (\pm) 0.02(6)</td>
<td>0.29 (\pm) 0.07(5)</td>
</tr>
<tr>
<td>15</td>
<td>0.41 (\pm) 0.03(6)</td>
<td>0.30 (\pm) 0.14(5)</td>
</tr>
<tr>
<td>19</td>
<td>1.40 (\pm) 0.13(5)</td>
<td>1.10 (\pm) 0.22(6)</td>
</tr>
<tr>
<td>20</td>
<td>1.27 (\pm) 0.29(7)</td>
<td>1.44 (\pm) 0.36(6)</td>
</tr>
<tr>
<td>21</td>
<td>0.70 (\pm) 0.33(6)</td>
<td>0.31 (\pm) 0.21(6)</td>
</tr>
</tbody>
</table>

Data are mean values \(\pm\) SE; no. in parenthesis is no. of embryos. \(\Delta\), Change in parameter. There were no significant differences between prazosin and phentolamine injections.

---

**Figure 5. Absolute change in embryonic MAP (A) and \(f_H\) (B) caused by the injection of an \(\alpha\)-blocker (1 mg/kg; see text). An asterisk indicates significant response to drug injection \((P \leq 0.0167)\). Like letters represent similar responses between days. Data are plotted as mean absolute change \(\pm\) SE.**

---

**Table 5. Plasma catecholamine concentrations at each day of incubation tested**

<table>
<thead>
<tr>
<th>Day</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>65.0 (\pm) 8.5*</td>
<td>2.3 (\pm) 0.3*</td>
<td>3.2 (\pm) 0.4*</td>
</tr>
<tr>
<td>15</td>
<td>55.7 (\pm) 12.6*</td>
<td>2.4 (\pm) 0.8*</td>
<td>1.1 (\pm) 0.3*</td>
</tr>
<tr>
<td>19</td>
<td>169.4 (\pm) 52.0†</td>
<td>80.5 (\pm) 1.0†</td>
<td>5.4 (\pm) 0.8†</td>
</tr>
<tr>
<td>20</td>
<td>137.0 (\pm) 15.9†</td>
<td>71.2 (\pm) 8.8†</td>
<td>9.1 (\pm) 1.3†</td>
</tr>
<tr>
<td>21</td>
<td>97.3 (\pm) 40.2*</td>
<td>51.2 (\pm) 19.9†</td>
<td>3.2 (\pm) 1.0†</td>
</tr>
</tbody>
</table>

Data (in ng/ml) are mean values \(\pm\) SE; no. of embryos. Like symbols indicate similar effects of drug injection between developmental days.
attributed to inappropriate doses of atropine or the insensitivity of muscarinic receptors given the successful elimination of responses to acetylcholine via pretreatment with atropine (Fig. 1).

Two additional experiments corroborate the lack of vagal tone during ontogeny in chickens. First, hexamethonium, which blocks nerve transmission at the ganglionic level, had no effect on heart rate; thus no autonomic tone (parasympathetic or sympathetic) was present. If any tonic activity was present (most likely of parasympathetic origin; Ref. 12), hexamethonium would remove this tone and heart rate would presumably rise.

The second experiment utilized hypoxia as an acute cardiovascular stress to address the possibility of acute vagal activation, even if a tonic function was lacking. Embryonic exposure to 10% O₂ produced a general cardiovascular depression on all days of incubation studied (Fig. 3), a response that was unaltered by atropine injection. This finding is in general agreement with previous studies on embryonic chickens (29), suggesting that the vagus plays no role in cardiovascular control. This contrasts with the hypoxic response of adult chickens (4, 17) and fetal sheep, which depend largely on changes in vagal activity to modulate cardiovascular function (11). Collectively, this study shows that normal cardiovascular development occurs in the absence of tonic vagal input in embryonic chickens until external pipping.

In contrast to the lack of cholinergic tone, the bradycardic response to an injection of propranolol revealed a clear β-adrenergic tone that is important for the maintenance of basal chronotropic activity. Although the heart rate response of embryonic chickens to β-blockade (26, 30) is similar to that of adults (cf. Ref. 4), the participation of sympathetic efferents in cardiac regulation is unlikely in embryos (12, 23). Therefore, the adrenergic tone is presumably related to the increasing levels of circulating catecholamines (Table 5). Further evidence is provided by the lack of action of hexamethonium that, as previously indicated, blocks postganglionic autonomic transmission.

The catecholamine levels reported here are consistently higher than those determined in an earlier study (7) while being similar to those reported on days 19–21 in a second study (33). Because blood was obtained by hemorrhage, which can trigger the release of catecholamines (unpublished observations), the plasma levels measured here may indicate the capacity for release under cardiovascular stress rather than the resting levels in the plasma. Regardless of the capacity for catecholamine release into the plasma, embryonic chickens do not exhibit a concomitant increase in cardiac reactivity to propranolol, which might be instrumental in preventing a supramaximal stimulation of the heart. Over the period of 17 to 19 days of incubation the pacemaker tissue, as well as the ventricle, is subsensitive to catecholamines (12, 20), probably because of the saturation of the receptors, downregulation, or desensitization. The peak activity of catechol-α-methyl transferase and monoamine oxidase (catabolic enzymes responsible for the inactivation of catecholamines) in cardiac tissue of chickens also occurs on days 19–20 (14). Collectively, these studies may explain the constant cardiac response to propranolol in the presence of increasing plasma catecholamines. Thus the experimental evidence indicates that humoral catecholamines play a role in tonic cardiac regulation, although the specific details on how this is achieved require further research.

Vascular regulation: α- and β-adrenergic tones. Given that extraembryonic vascular beds lack autonomic innervation and that the innervation of intraembryonic vasculature is nonfunctional, vascular regulation during development must be based primarily on humoral factors such as catecholamines.

In view of these facts, changes in arterial pressure after the injection of an α- or β-antagonist indicate a dependence on different populations of adrenergic receptors for vascular regulation. After propranolol injection, chicken embryos exhibited a clear hypertension on days 19 and 20 (26, 30), opposite to the hypotension occurring in adult chickens (4, 17). These different pressure responses are likely related to the presence of a chorioallantoic vascular bed in embryos, a structure that has been suggested to be analogous to the mammalian placenta (21). If the chorioallantoic vasculature has an active population of β-adrenergic receptors, as has been suggested for the mammalian placenta (5), it could result in an accentuated β-adrenergoreceptor vaso-dilation in embryonic chickens. Thus the negative inotropic action, which typifies the adult cardiac reaction to β-blockade, may be masked by an overriding chorioallantoic vasoconstriction in embryonic chickens, resulting in hypertension. Such a dilatatory tone may have important consequences for the regulation of blood oxygenation during chicken development.

In contrast to the primary cardiac actions of β-antagonists, injections of α-antagonists revealed strong α-adrenergic tone on the vasculature (Fig. 5A). Prior studies showed similar hypotensive results after α-blockade in embryonic chickens from day 6 to day 16 (18, 26, 30). Qualitatively, results with either α-blocking agent were similar, suggesting that the α₁-receptor is the primary α-subunit responsible for maintaining vascular tone in embryonic chickens.

In addition to the vascular responses, α-adrenergic blockade also had a negative effect on cardiac activity. Given the action of phenolamine on cardiac function in adult mammals, the possible contribution of cardiac α-adrenergic receptor blockade to the decrease in embryonic heart rate must be acknowledged (8). However, it is plausible that the negative chronotropic action of α-blockade could be secondary to the general vasodilation, resulting in a reduced venous return to the heart caused by blood pooling in the extraembryonic circulation. Determination of the changes in vascular resistance after selective α-blockade are needed to establish the origin of any negative chronotropic action.

As illustrated in Fig. 5A, day 19 and 20 embryos exhibited the highest reactivity to α-blockade whereas day 21 embryos were unaffected by drug treatment.
This response indicates that, as previously suggested (10), there is a dependence on α-adrenergic tone to maintain basal cardiovascular function that is maximal just before internal pipping and lung ventilation. Similar results have been obtained in fetal sheep, which show an increase in α-adrenergic tone with a magnitude that peaks at the end of gestation (2). Such a late gestational dependence on adrenergic receptors is essential for the maintenance of fetal cardiovascular performance during the asphyxia associated with parturition (16, 19). This dependence then decreases dramatically in the neonate after parturition and resumption of normoxic blood PO₂ associated with lung ventilation. An analogous event may explain the peak and subsequent drop in α-adrenergic tone late in chicken ontogeny. As the embryonic gas exchange organ regresses, an increased α-adrenergic tone may ensure proper delivery of oxygen to embryonic tissues. Therefore, the peak in α-blockade response on day 19 of incubation may represent a heightened dependence on adrenergic systems to protect against reduced oxygen supply, and this is simultaneous with the increased capacity for catecholamine release (Table 5).

A surge in catecholamine levels at parturition is also part of the mammalian ontogenetic repertoire that has been implicated to participate in the process of absorption of lung fluid as well as maintenance of glucose supply to the heart besides sustaining peripheral resistance (16). Thus it is conceivable that in chickens catecholamines are involved in the timing of internal pipping by regulating the blood flow to the extraembryonic circulation and ensuring adequate perfusion pressures to maintain gas exchange. As the CAM begins to regress, before the onset of lung ventilation, the tonic contribution of α-adrenoceptors may be important for maintaining CAM perfusion. In addition, the established increase in catecholamine concentration may be instrumental in the improvement of blood oxygenation via an increase in synthesis of carbonic anhydrase and 2,3-diphosphoglycerate (7).

Perspectives

This study has delineated the basic mechanisms of cardiovascular control in embryonic chickens, which could be extended to the cardiovascular development of other vertebrate groups. Data from the present study indicate that vagal tone is absent throughout the ontogeny of chicken embryos. In addition, this work has established that the capacity for catecholamine release exhibits profound maturational changes during chicken ontogeny. This developmental change in catecholamine release ability is coupled to a constant β-adrenergic tone on the heart and an α-adrenergic tone on the vasculature that increases in magnitude as development progresses.

The absence of a clear vagal tone during embryonic development is not unique to the chicken. Fetal sheep have been shown in several studies to possess limited or no vagal tone during the latter third of gestation (24, 31, 32), possibly indicating that a lack of vagal tone is genetically dictated. An equally appealing alternative explanation is that the maturation of vagal tone is inversely related to the degree of maternal care, with embryos that are more exposed to environmental fluctuations showing a functional vagal tone on the heart earlier in development.

Even if vagal input is not critical to normal cardiovascular regulation during development, the adrenergic system is clearly necessary. The correlation between catecholamine concentrations and peak in cardiovascular sensitivity further supports their importance in basal cardiovascular function of embryonic chickens. Similar characteristics are apparent in fetal sheep, which exhibit predominantly α-adrenergic activity with catecholamine levels peaking at term (2, 11, 24). Collectively, these data suggest that embryonic vertebrates depend on hormonal adrenergic influence to control cardiovascular activity and possibly to ensure successful embryonic life. Further work is needed to delineate the degree to which these patterns are reflected in other groups.

We are greatly indebted to Gunilla Rydgren (Zoophysiology, University of Göteborg) for the HPLC analysis of catecholamine. J. Altimiras was a recipient of a postdoctoral fellowship from the Danish Research Council. D. Crossley was supported by National Science Foundation Grant IBN-9616138 to W. W. Burggren and the Danish Center for Respiratory Adaptation. Present address of J. Altimiras: Dept. of Zoophysiology, Univ. of Göteborg, Box 463, SE 405 30 Göteborg, Sweden.

REFERENCES


