

**α-Adrenergic contribution to the cardiovascular response to acute hypoxemia in the chick embryo**

A. L. M. Mulder, C. A. Van Goor, D. A. Giussani, and C. E. Blanco. α-Adrenergic contribution to the cardiovascular response to acute hypoxemia in the chick embryo. Am J Physiol Regulatory Integrative Comp Physiol 281: R2004–R2010, 2001.—Fetal responses to acute hypoxemia include bradycardia, increase in blood pressure, and peripheral vasoconstriction. Peripheral vasoconstriction contributes to the redistribution of the cardiac output away from ancillary vascular beds toward myocardial, cerebral, and adrenal circulations. We investigated the effect of α-adrenergic receptor blockade on this fetal response. Fluorescent microspheres were used to measure cardiac output distribution during basal and hypoxic conditions with and without phentolamine treatment. Phentolamine altered basal cardiac output distribution, indicating a basal α-adrenergic tone, but this was mainly noted at the earlier stages of incubation. During hypoxemia, phentolamine prevented vasoconstriction in the carcass. At day 19 of incubation, the percent cardiac output distributed to the carcass increased by 20% compared with a decrease in the control group by 17%. Phentolamine markedly attenuated the subsequent redistribution of the cardiac output toward the brain (from +102% in the control group to −25% in the phentolamine-treated group) and the heart (from +196% in the control group to +69% in the phentolamine-treated group). In the chick embryo, α-adrenergic mechanisms contribute to the maintenance of basal vascular tone and to the redistribution of the cardiac output away from the peripheral circulations toward the brain and heart during hypoxic conditions.

The physiological mechanisms mediating these cardiovascular responses involve neural and endocrine components. Studies in fetal sheep in late gestation have shown that the initial peripheral vasoconstriction in response to hypoxemia is part of a carotid chemoreflex that is mediated via α-adrenergic efferent pathways, inasmuch as it can be abolished by carotid sinus nerve section (2, 18) or by α-adrenergic receptor blockade with phentolamine (18). Once the carotid chemoreflex vasoconstriction is triggered, endocrine agents such as catecholamines (22), arginine vasopressin (15), angiotensin II (20), and neuropeptide Y (12) are released into the fetal circulation to maintain peripheral vasoconstriction throughout the duration of the hypoxic challenge.

Studies in fetuses of other species have shown that the contribution of neuroendocrine mechanisms to the fetal cardiovascular responses to acute stress may be modified by the intrauterine environment. For example, the fetal llama, a species adapted to the chronic hypobaric hypoxia of life at altitude (27), shows an intense peripheral vasoconstrictor response to acute hypoxemia that is mediated by upregulated α-adrenergic mechanisms (16).

The redistribution of the cardiac output in response to acute hypoxemia has also been documented in the chick embryo in the last half of the incubation period (29). The cardiovascular responses to acute hypoxemia in the chick embryo show a developmental pattern, with peripheral vasoconstriction becoming progressively more intense by day 19 of incubation (hatching = 21 days) (29). Additional studies from our laboratory have reported high concentrations of circulating catecholamines in response to acute hypoxemia in the chick embryo relative to those measured in the sheep fetus at comparable stages of gestation or incubation (28). In addition, this plasma catecholaminergic response to acute hypoxemia also becomes progressively larger with advancing incubation time (28), and in vitro studies, using femoral arteries isolated from chick embryos, have shown pronounced α₁-adrenergic contractile responses that also increase from day 15 to day 21.
19 of incubation (25). Therefore, previous studies largely support the hypothesis that, in the chick embryo, peripheral vasoconstriction and redistribution of the cardiac output away from ancillary circulations are highly dependent on α-adrenergic pathways. In the present study, we have tested this hypothesis by investigating the effects of treatment of the chick embryo with the α-adrenergic receptor antagonist phentolamine on the redistribution of the cardiac output in response to acute hypoxemia at different stages of incubation.

METHODS

Preparation. Fertilized eggs of White Leghorn chickens were maintained in a commercial incubator at 38°C and 60% humidity. At the desired incubation time, the eggs were transferred to a clinical infant incubator and catheterized as previously described in detail (30). Briefly, eggs were opened at the air cell and placed in a holder within a Plexiglas box. A polyethylene catheter stretched by heat to a diameter of 100 μm was inserted into a chorioallantoic vein. Clay was used to fix the catheter to the eggshell. Later, the catheter was used for injections of fluorescent microspheres and phentolamine or saline solution. Throughout the procedure, the O₂ concentration in the box was maintained by supplied mixtures of warmed and humidified N₂ and O₂, delivered at a constant flow of 5 l/min.

Experimental protocol. Sixty chick embryos were included in the study. At days 11, 15, and 19 of incubation, 10 chick embryos were randomly assigned to a control group and 10 to an experimental group. Cardiac output distribution was measured by injection of 15-μm fluorescent microspheres (Fluospheres, Molecular Probes, Eugene, OR) suspended in saline and 0.05% Tween 80 (1,000,000 spheres/ml). In each control and each experimental group, at each stage of incubation, 0.04 ml (40,000 spheres) of the suspension of blue-green fluorescent microspheres were injected for measurement of basal cardiac output distribution. Thereafter, each control group was injected with saline (0.9% NaCl), and each experimental group was treated with phentolamine (Sigma Chemical; 2.5 μg/g in 5 μl/g embryo). After 5 min, 40,000 orange fluorescent microspheres were injected to determine the effect of α-adrenergic receptor blockade on basal cardiac output distribution. After 1 min, acute hypoxemia was induced in the chick embryo by changing the supplied gas mixture to 100% N₂ for 5 min. Previous studies have shown that this regimen results in a fall in the arterial P₂O₂ of the chick embryo from 5.11 ± 0.38 to 1.20 ± 0.21 kPa (28). After 5 min of hypoxemia, 40,000 crimson fluorescent microspheres were injected to determine the effect of α-adrenergic receptor blockade on cardiac output distribution during the hypoxic challenge. At the end of the experiment, 5 min after normoxia was reestablished, all chick embryos were decapitated and the chorioallantoic membrane (CAM), brain, heart, lungs, intestine, liver, and yolk sac were dissected for measurement of microsphere distribution (28). All experiments complied with the Dutch law for animal experimentation.

Measurement of microsphere distribution. Organs and the remaining carcass were digested in test tubes in a 2 M ethanol-KOH solution. The microspheres were isolated from the homogenate by centrifugation, a method shown to result in recovery of ~100% of microspheres (36). The dye was extracted with 3 ml of 2-(2-ethoxyethoxy)ethylacetate, and the fluorescence was measured by fluorometry using a fluorospectrometer (model LS-50B, Perkin-Elmer). No correction for spectral overlap was used, since the excitation and emission spectra of the three dyes were well separated. The fraction of cardiac output that was directed to the tissue was expressed as the level of the fluorescence of the sample, corrected for background, divided by the sum of fluorescence of all tissues (28).

RESULTS

Basal cardiac output distribution. Baseline data for cardiac output distribution are shown in Fig. 1. The data are from control and experimental groups (n = 20 per incubation period) before the injection of saline or phentolamine. Cardiac output was largely distributed to the CAM and the carcass during basal conditions at all stages of incubation studied (Fig. 1). With increasing incubation time, the fractions of the cardiac output directed to the heart, lungs, brain, intestine, and carcass increased, and those directed to the yolk sac and CAM decreased (Fig. 1).

Effect of α-adrenergic receptor blockade on basal cardiac output distribution. At day 11, treatment of the chick embryo with phentolamine during basal conditions led to an increase in the fraction of the cardiac output directed to the brain, heart, carcass, and intestines but no change in the fraction of the cardiac output directed to the liver (Fig. 2). At day 15, although the fraction of the cardiac output to the carcass still increased after phentolamine treatment during basal conditions, the increases to the heart and intestines fell outside significance, and, in contrast to measurements at day 11, there was a fall in the fraction of the cardiac output distributed to the brain and liver. At day 19 of incubation, there was no significant increase in the fraction of the cardiac output directed to any organ,
and the fall in cardiac output distribution to the brain and liver persisted after treatment with phentolamine during basal conditions (Fig. 2).

**Effect of α-adrenergic receptor blockade on cardiac output distribution during acute hypoxemia.** With advancing incubation time, cardiac output was preferentially distributed to the brain and heart at the expense of the liver, yolk sac, intestines, and carcass during acute hypoxemia (Fig. 3). The percent changes in cardiac output distribution are summarized in Table 1. At day 11, treatment of the chick embryo with phentolamine during acute hypoxemia diminished the increase in the fraction of cardiac output directed to the lungs, reversed the increase in cardiac output to the brain and liver, reduced the fall in cardiac output to the yolk sac, and led to an increase in the fraction of cardiac output directed to the carcass (Fig. 3). At day 15, treatment of the chick embryo with phentolamine during hypoxemia produced changes similar to those measured at day 11, except the fraction of cardiac output directed to the heart was significantly diminished (Fig. 3). The greatest effect of treatment with phentolamine during acute hypoxemia occurred at day 19 of incubation, when the fraction of cardiac output directed to the heart was substantially reduced and there was a pronounced reversal in the fraction of cardiac output directed to the brain, intestines, and carcass.

**DISCUSSION**

The present study in the chick embryo shows that 1) sympathetic α-adrenergic tone plays an important role in the distribution of cardiac output under basal conditions, 2) increased sympathetic α-adrenergic tone is largely responsible for the redistribution of cardiac output in response to acute hypoxemia, and 3) the contribution of the sympathetic α-adrenergic tone toward mediating the redistribution of cardiac output away from the carcass toward the heart and brain during acute hypoxemia increases with advancing incubation time.

The chick embryo, as an experimental model, offers the primary advantage to study the development of fetal cardiovascular responses to adverse conditions without the influence of maternal vasoactive factors. This is not the case for mammalian species, where, for instance, maternal corticosteroids released in response to hypoxemia might easily cross the placenta and influence the fetal cardiovascular responses to the challenge (3). On the other hand, the chick embryo experimental model also has some limitations. For example, it is not possible to obtain repetitive blood samples or to measure the absolute cardiac output and regional blood flows, primarily because of the small size of the embryos and the limited blood volume.

In the chick embryo, the presence of vascular α- and β-adrenergic receptors has been reported from day 7 of incubation (13). In addition, intravenous injection of epinephrine and norepinephrine evokes an increase in heart rate and blood pressure in the chick embryo from day 7 of incubation (14). Adrenal medulla cells arise from the sympathetic chains on day 5 of incubation (8), and catecholamines are detected in very small amounts from that day in allantoic fluid (4). In the adrenal gland, epinephrine concentrations are relatively low until day 15, after which they increase markedly toward hatching (37). Catecholamine concentrations in plasma have been reported in the chick embryo from day 6 of incubation (10). Previous studies in the chick embryo from our laboratory have described an ontogenic increase in catecholamine release in response to acute hypoxemia (28) that parallels the mat-
Fig. 3. Effect of α-adrenergic receptor blockade on cardiac output redistribution in response to acute hypoxemia. Bars, means; error bars, SE. *Significant difference (P < 0.05) compared with baseline.
Table 1. Percent change in cardiac output distribution in response to acute hypoxia for phentolamine-treated chick embryos and controls on days 11, 15, and 19 of incubation

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Phentolamine</th>
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<tr>
<td></td>
<td>Heart</td>
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<tr>
<td></td>
<td>59.5</td>
<td>44.3</td>
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<tr>
<td></td>
<td>(46.6 to 107.7)</td>
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<td></td>
<td>Lungs</td>
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<td></td>
<td>187.0</td>
<td>62.5*</td>
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<td></td>
<td>(90.1 to 324.4)</td>
<td>(21.9 to 138.6)</td>
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<tr>
<td></td>
<td>Brain</td>
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<td></td>
<td>61.6</td>
<td>-18.1*</td>
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<tr>
<td></td>
<td>(12.1 to 107.7)</td>
<td>(-31.8 to 9.3)</td>
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<td></td>
<td>CAM</td>
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<td></td>
<td>16.3</td>
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<td></td>
<td>(8.6 to 26.8)</td>
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<td></td>
<td>Liver</td>
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<tr>
<td></td>
<td>-16.1</td>
<td>-32.7*</td>
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<td></td>
<td>(-25.4 to 32.4)</td>
<td>(-39.9 to -21.8)</td>
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<td></td>
<td>Intestine</td>
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<td></td>
<td>53.6</td>
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<td></td>
<td>(42.4 to 90.1)</td>
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<td></td>
<td>Yolk sac</td>
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<td></td>
<td>-85.8</td>
<td>-35.2*</td>
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<td></td>
<td>(-89.8 to -78.5)</td>
<td>(-70.7 to 24.0)</td>
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<td></td>
<td>Carcass</td>
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<td></td>
<td>6.5</td>
<td>21.9*</td>
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<td></td>
<td>(1.3 to 7.8)</td>
<td>(12.3 to 64.5)</td>
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Values represent median change in cardiac output directed to each organ expressed as percentage of baseline level, with interquartile range (25th–75th percentile) in parentheses; CAM, chorioallantoic membrane. *P < 0.05 vs. control.

The concept that the vasoconstrictor contribution of sympathetic α-adrenergic tone is greater in the carotid and femoral circulations under basal conditions earlier than later in gestation. However, the comparison between experiments in fetal sheep and fetal llamas may not be justified, particularly since the contribution of the α-adrenergic system is upregulated in the llama (16). Alternatively, the lack of a significant increase in the fraction of cardiac output directed to the carcass after phentolamine at day 19 of incubation may represent the maturation of α-adrenergic-independent vasoconstrictor mechanisms that act to reduce blood flow in this circulation, even in the absence of α-adrenergic influences. A possible candidate may be angiotensin II, which has been reported to induce a greater pressor response under basal conditions in fetal sheep near term than earlier in gestation (34).

Response to acute hypoxia. In the present study, treatment of the chick embryo with phentolamine during acute hypoxemia completely prevented the redistribution of cardiac output to favor of the brain at all stages of incubation studied. Given the proportion of cardiac output distributed to the carcass after phentolamine treatment was smaller than at previous stages of incubation. In combination, these data suggest that the contribution of the α-adrenergic vascular tone under basal conditions becomes less important with progression of the incubation period. Comparable data from studies in the fetal llama show that α-adrenergic blockade with phentolamine at 0.6–0.7 gestation resulted in significant increases in carotid and femoral artery blood flows (16) under resting conditions. The same treatment in 0.8-gestation fetal sheep had no effect on basal carotid or femoral blood flows (18). Together, these findings support the concept that the vasoconstrictor contribution of sympathetic α-adrenergic tone is greater in the carotid and femoral circulations under basal conditions earlier than later in gestation.
mechanisms other than those promoting α-adrenergic-mediated peripheral vasoconstriction. Such mechanisms may include β2-adrenergic receptor stimulation (11) and local nitric oxide release induced by hypoxemia, as shown in fetal sheep (31).

One study in late-gestation fetal sheep reported that blood flow to the heart increased during acute hypoxemia, but in contrast to the present study, myocardial blood flow increased further after fetal treatment with phenoxybenzamine, another α-adrenergic receptor antagonist (32). In that study, blood flow to the heart was measured ~20 min after the onset of hypoxia in the presence of phenoxybenzamine. Therefore, it is likely that the enhancement of myocardial blood flow after treatment of the sheep fetus with the α-adrenergic antagonist may have been due to prolonged hypoxemia with developing acidemia, promoting a greater recruitment of local vasodilator mechanisms. An alternative explanation is that the sheep fetus may be more dependent than the chick embryo on local vasodilator mechanisms to promote an increase in myocardial blood flow during acute hypoxemia.

Interestingly, in the present study, treatment of the chick embryo with phentolamine had a progressively greater effect on the distribution of the cardiac output to the carcass during acute hypoxemia with advancing incubation time. Therefore, although treatment with phentolamine during hypoxemia enhanced carcass blood flow at day 11, it mildly reversed the fall in carcass blood flow at day 15, and it markedly reversed this fall at day 19. Previously, we reported an ontogenic increase in the magnitude of the peripheral vasoconstriction during acute hypoxemia (29) and that these changes paralleled the maturation of the plasma catecholaminergic response to acute hypoxemia in the chick embryo (28). Past data, together with the results of the present study, therefore suggest a progressively larger contribution of sympathetic α-adrenergic neuroendocrine mechanisms mediating peripheral vasoconstriction during acute hypoxemia as the chick embryo approaches hatching. Maturation of this sympathetic α-adrenergic-mediated vasoconstriction may reflect the development of an important defense mechanism that helps redistribute cardiac output away from the peripheral vessels toward the heart when under adverse conditions during incubation in the chick embryo.

Perspectives

In the sheep fetus, α-adrenergic activity may be increased by neural and endocrine pathways. For example, increased sympathetic discharge, such as that induced by hypoxemia, results in norepinephrine release from sympathetic nerve endings (1). Sympathetic stimulation of the splanchnic nerve also results in catecholamine release from the adrenal medulla (7, 9). In addition, hypoxia might stimulate the adrenal gland to release catecholamines into plasma by a direct effect on chromaffin cells (33). Jones et al. (23) showed that in fetal sheep the increase in plasma epinephrine was totally abolished and that the increase in norepinephrine was reduced 90% in response to hypoxemia after adrenal demedullation. This suggests that in fetal sheep the increase in circulating plasma catecholamines in response to hypoxemia originates primarily in the adrenal medulla. Furthermore, in the sheep fetus, the direct effect of hypoxia on epinephrine release from the adrenal gland becomes less important toward full term, when sympathetic innervation of the adrenal gland is complete (5, 6). The present study showed that, in the chick embryo, phentolamine abolished the fall in carcass blood flow in response to hypoxemia and that this effect became progressively more marked as the chick embryo approached full term. However, the present study cannot discriminate whether the maturation of the α-adrenergic activity mediating the ontogenic increase in the peripheral vasoconstrictor response to acute hypoxemia in the chick embryo is of neural and/or endocrine origin, since phentolamine will antagonize both mechanisms. The origin of the increased α-adrenergic activity during acute hypoxemia as the chick embryo approaches full term is therefore unresolved and will be the subject of subsequent investigations.

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REFERENCES