Pulmonary artery endothelium-dependent vasodilation is impaired in a chicken model of pulmonary hypertension

Luis A. Martinez-Lemus, R. Kelly Hester, Elizabeth J. Becker, Joan S. Jeffrey, and Ted W. Odom. Pulmonary artery endothelium-dependent vasodilation is impaired in a chicken model of pulmonary hypertension. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R190–R197, 1999.—Among chicken strains, broilers are prone to pulmonary hypertension, whereas Leghorns are not. Relaxations to endothelium-dependent (ACh, A23187) and endothelium-independent [sodium nitroprusside (SNP), papaverine (PPV)] vasodilators were compared in preconstricted pulmonary artery (PA) rings from these chicken strains. ACh (10⁻⁷, 10⁻⁶, and 10⁻⁵ M) and A23187 (10⁻⁶ and 10⁻⁵ M)-induced relaxations were smaller (P < 0.05) in broilers than in Leghorns. N⁶-nitro-L-arginine methyl ester (10⁻⁵ M) caused similar reductions in ACh-induced relaxations in both strains. L-Arginine (10⁻⁴ M) enhanced ACh-induced relaxations more in broilers than in Leghorns. Relaxations to 10⁻⁸–10⁻⁴ M SNP did not differ between strains, but were greater (P < 0.05) in broilers than in Leghorns at higher concentrations (10⁻⁵ and 10⁻⁴ M). PPV (10⁻⁴ M)- and SNP (10⁻⁴ M)-induced relaxations were greater in Leghorns than in broilers at 10⁻⁴ M and higher concentrations (176.2 ± 10.7% vs. 120.9 ± 14.7% and 201.3 ± 7.8 vs. 171.2 ± 10.7%, respectively, P < 0.05). Broiler PA rings appear to have increased intrinsic tone and reduced endothelium-derived nitric oxide activity, both of which may contribute to the susceptibility of broiler chickens to pulmonary hypertension.

broiler chicken; Leghorn chicken; nitric oxide; intrinsic tone

ENDOTHELIUM-DERIVED NITRIC oxide (EDNO) is actively involved in the control of vascular tone in both the systemic and pulmonary circulations. In general, the role of EDNO is to reduce vascular tone under basal conditions, as well as in response to various endogenous, mechanical, and pharmacological stimuli (1, 5). However, basal synthesis and activity of EDNO in the pulmonary circulation is controversial in several animal species (2, 3, 20). EDNO is produced in the endothelial cell by both the constitutive and the inducible form of the enzyme nitric oxide (NO) synthase (NOS; Ref. 29). NOS forms EDNO from the guanido nitrogen of the amino acid L-arginine (26). Once produced, EDNO readily diffuses into the vascular smooth muscle where it activates soluble guanylate cyclase. This activation results in an increased concentration of guanosine 3',5'-cyclic monophosphate, which in turn induces vascular smooth muscle relaxation and vasodilation (15).

Extensive research suggests that significant changes in EDNO synthesis occur in pulmonary hypertension (2, 9, 12, 14, 19, 20, 32). However, whether these changes involve an increased or a decreased synthesis of EDNO is unclear, specifically because conflicting responses to EDNO agonists have been reported in pulmonary hypertensive animals between species and within a species. Reports demonstrating increased relaxation responses to EDNO agonists and an increased expression of NOS in the pulmonary vasculature of rats exposed to monocrotaline and hypoxia suggest that EDNO synthesis is increased during pulmonary hypertension (32, 33). Others, however, have suggested that a reduced expression of NOS and a reduced production of NO are in part responsible for the pulmonary hypertension observed in animals exposed to hypoxia (2, 12, 20, 22). The cause of these contradictory results is unclear, but they may be due to differences in NOS expression, the use of different animal species, differences in the protocols used to induce pulmonary hypertension, or variations in testing for pulmonary vasoactivity.

Among chickens, meat-producing broiler strains are highly prone to severe pulmonary hypertension and congestive right heart failure (16). In comparison, egg-producing Leghorn strains are highly resistant to severe pulmonary hypertension under both normoxic and hypoxic conditions (23). The increased susceptibility to pulmonary hypertension of the broiler chicken is believed to be the inadvertent consequence of selective breeding for rapid body growth and better feed conversion. It has been proposed that this selective breeding has resulted in anatomic and functional inadequacies of the pulmonary circulation in the broiler chicken (27, 42). Indeed, a comparison between unselected wild chickens and broiler chickens indicated that after a similar increase in pulmonary arterial pressure in response to acute unilateral pulmonary artery occlusion, unselected chickens but not broiler chickens were able to reduce pulmonary vascular resistance and pulmonary arterial pressure to levels not different from those before the occlusion (45, 48). In addition, increases in dietary L-arginine have been shown to reduce the incidence of severe pulmonary hypertension in broiler chickens (46, 47). These data suggest that the vasodilator capacity of the pulmonary circulation in the broiler chicken is impaired and that an increased availability of L-arginine, the NOS substrate, is restorative.
We tested this hypothesis using two strains of chickens with different susceptibilities to pulmonary hypertension, namely Leghorn and broiler chickens. The disparity of susceptibility to pulmonary hypertension between these two strains provides a unique model to study and relate pulmonary artery (PA) vasoactivity with pulmonary hypertension in individuals from the same species without the use of hypoxia or lung injury. Thus, in the present study, we compared preconstricted PA rings from broiler and Leghorn chickens for their ability to relax in response to several endothelium-dependent and endothelium-independent vasodilators in the absence or presence of either L-arginine or an NOS inhibitor.

METHODS

Animals. Male 1-day-old slow-growing single comb White Leghorn and fast-growing broiler chickens were acquired from Hyline Hatchery (Hyline, Bryan, TX) and Ideal Hatchery (Cameron, TX), respectively. From 1 day of age, all chickens were kept under a 24-h light regimen in a temperature-controlled Petersime brooder unit (Petersime Incubator, Gettysburg, OH) and provided ad libitum access to water and a standard corn-soybean starter ration (3,190 kcal metabolized energy/kg of diet, 22% crude protein, and 1.62% arginine) formulated to meet or exceed requirements of the National Research Council for broilers (25). The use of chickens in all experimental protocols was approved by Texas A&M University’s Animal Care Committee.

Dissection. Chickens 1 to 3 wk of age were weighed after euthanasia by cervical dislocation. The hearts and lungs were exposed via thoracotomy, and the pericardial sac was removed and placed in cold aerated buffer solution. Excessive connective tissue was carefully removed from the PA section, and a ring 3 mm in length was carefully cut with dissecting scissors.

The heart was also removed, and the ventricles were dissected from the atria along with the atrioventricular fat. The free wall was then dissected from the left ventricle. Immediately after dissection, wet weights of both ventricles were obtained, and the ratio of right ventricular free wall weight to total ventricular weight was calculated as an index of right ventricular hypertrophy and pulmonary hypertension (8).

Equilibration. PA rings were suspended between two stainless steel hooks and vertically mounted in a 20-ml water-jacketed organ bath containing bicarbonate buffer solution (in mM: 142 NaCl, 5.4 KCl, 11.0 dextrose, 2.0 CaCl2, 1.2 MgCl2, and 18.0 NaHCO3, pH 7.40). Phentolamine (10–5 M) was added to the buffer solution to reduce any effects on α-adrenoceptors by β-adrenoceptor agonists or catecholamines potentially released by KC1-induced contractions (4, 24). The buffer solution was continually aerated with 95% O2 and 5% CO2 and maintained at a normal chicken temperature of 41°C using a circulating water bath. The lower hook was attached to a stainless steel rod mounted within the organ chamber, whereas the upper hook was connected to the lever of a force-displacement transducer (Grass FT.03, Grass Instrument, Quincy, MA) to record isometric contractions. Increments in force were quantified as millimeter deflections recorded by a polygraph (model 79A, Grass Instrument).

Before each experiment, transducers were calibrated so that each 20-mm deflection was equal to 1 g of force.

All PA rings were allowed to equilibrate for 2 h in the organ bath buffer solution while maintaining an optimal preload tension of 1 g, as determined in preliminary length-tension experiments (data not shown). During the equilibration period, the buffer solution was replaced every 10–15 min, and the baseline was readjusted as needed. Two KC1-induced vascular constrictions were induced within the 2-h equilibration period in the following manner. Thirty minutes after the onset of the equilibration period, PA rings were constricted with a buffer solution containing an additional 40 mM KC1 (45.4 mM total) substituted equimolar for NaCl of the original buffer solution. Maximum constriction was reached within 10–15 min, at which time the KC1 buffer solution was rinsed out and replaced with the original buffer solution. The PA rings were incubated another 30 min before a second KC1 constriction was elicited. After this second constriction and rinse, the PA rings were incubated for an additional 40 min before the experimental protocol was begun.

Vasodilator response to ACh, calcium ionophore A23187, and sodium nitroprusside. Individual concentration-response relationships for all vasodilator agents were completed in a cumulative manner without any intervening washout of bath chambers. After the equilibration period, the PA rings were submaximally constricted with a 75% (76 ± 7.2%)-effective concentration of endothelin-1 (ET-1, 10–7–2 M). This concentration of ET-1 was determined by concentration-response relationships performed in preliminary studies (data not shown). Once constriction to ET-1 had reached a plateau, relaxation effects of the receptor-mediated endothelium-dependent vasodilator ACh (10–7, 10–6, and 10–5 M), the non-receptor-mediated endothelium-dependent vasodilator A23187 (10–7, 10–6, and 10–5 M), or the endothelium-independent vasodilator sodium nitroprusside (SNP; 10–10, 10–9, 10–8, 10–7, 10–6, 10–5, and 10–4 M) were measured. After the maximal relaxation response to the greatest concentration of each of the above vasodilators was reached, papaverine (10–4 M) was added to define the maximal relaxation attainable in each ring. To determine any potential involvement of prostanoids in these experiments, formation of vasoactive prostaglandins was prevented by adding indomethacin (10–5 M) 30 min before ET-1 constriction to a series of PA rings from both chicken strains.

Effects of N G-nitro-L-arginine methyl ester and L-arginine on endothelium-dependent vasodilatation to ACh. In another series of experiments, PA rings were pretreated with the structural analog of L-arginine and competitive inhibitor of NOS, N G-nitro-L-argininemethyl ester (L-NAME; 10–3, 10–2, and 10–1 M), or L-arginine (10–4 M, 15 min) after equilibration and before the addition of ET-1. Subsequently, relaxation responses to increasing concentrations of ACh and papaverine were obtained as described above.

Plasma L-arginine measurements. In some chickens, blood samples were obtained by venous puncture of the brachial (wing) vein immediately before euthanasia. Plasma was analyzed for L-arginine concentration by a fluorometric high-performance liquid chromatography method with an o-phthalaldehyde-2-pyridine carboxylic acid derivation (49).

Chemicals. ACh, A23187, ET-1, papaverine, SNP, and indomethacin were obtained from Sigma Chemical (St. Louis, MO). L-Arginine and L-NAME were purchased from Research Biochemicals (Natick, MA), and phentolamine was obtained from Ciba-Geigy (Summit, NJ). All drugs were dissolved in double-distilled H2O, except for A23187 and indomethacin, which were initially dissolved in ethanol. Final concentration of ethanol in the organ bath buffer solution never exceeded 0.15%.
Statistical analysis. All data are presented as means ± SE. Relaxation responses are expressed as a percent of the maximal ET-1 constriction. Unpaired Student’s t-test or analysis of variance was performed on data using the general linear model procedure of the Statistical Analysis System (34). Partitioning of multiple significantly different means was accomplished using Duncan’s multiple range test (38). Differences were considered significant at a P < 0.05.

RESULTS

Body weight, ventricular hypertrophy, and plasma L-arginine in the broiler and Leghorn strains. Physical characteristics of both strains of chickens at euthanasia are outlined in Table 1. Mean body weights at 1 day of age were 55.0 ± 2.5 and 38.0 ± 2.2 g for broiler and Leghorn chickens, respectively. On average, from 1 day of age to the time of euthanasia, the rate of weight gain in broiler chickens was 1.8 times greater than that of their Leghorn cohorts. The ratio of right ventricular free wall weight to total ventricular weight indicative of right ventricular hypertrophy and pulmonary hypertension (8) was also significantly greater in broiler than in Leghorn chickens, suggesting that pulmonary arterial pressure was increased in the broiler chickens. Plasma L-arginine concentrations however, were not significantly different between the two strains.

<table>
<thead>
<tr>
<th>Chicken Strain</th>
<th>Body Wt, g</th>
<th>(RV/TV) × 100</th>
<th>[L-Arg], µM/ml</th>
</tr>
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<tbody>
<tr>
<td>Broiler</td>
<td>249.6 ± 25.6*</td>
<td>22.0 ± 0.4*</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>(n = 51)</td>
<td>(n = 51)</td>
<td>(n = 51)</td>
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<tr>
<td>Leghorn</td>
<td>105.5 ± 32.8</td>
<td>19.5 ± 0.6</td>
<td>0.42 ± 0.04</td>
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<tr>
<td>(n = 31)</td>
<td>(n = 31)</td>
<td>(n = 12)</td>
<td></td>
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</tbody>
</table>

Values are means ± SE. Numbers in parentheses, number of chickens. RV/TV, right ventricular-to-total ventricular weight ratio; [L-Arg], L-arginine plasma concentration. Body wt and RV/TV were significantly greater in broiler compared with Leghorn chickens. *P < 0.05 vs. Leghorn.

Fig. 1. Relaxation responses to increasing concentrations of acetylcholine in endothelin-1 (3 × 10⁻⁸ M)-preconstricted pulmonary artery rings from broiler (n = 48) and Leghorn (n = 29) chickens. Data points are means ± SE. *P < 0.05 vs. Leghorn at the same acetylcholine concentration.

Effects of ACh, calcium ionophore A23187, SNP, and papaverine in isolated avian PA rings. Prevention of the formation of vasoactive prostaglandins by addition of 10 µM indomethacin 30 min before ET-1 vasoconstriction did not affect any of the responses to ACh, A23187, or SNP (data not shown). Vasodilator responses to ACh in broiler and Leghorn PA rings are shown in Fig. 1. PA rings from both broiler and Leghorn chickens demonstrated a dose-dependent relaxation to ACh after ET-1 constriction. However, the percent relaxation achieved at each ACh concentration was significantly smaller in broiler than in Leghorn PA rings. Similarly a reduced relaxation response in broiler PA rings was observed when A23187 was used, with an exception at the lowest concentration (10⁻⁹ M), which induced only a small relaxation in either strain (Fig. 2).

Fig. 2. Relaxation responses to increasing concentrations of calcium ionophore A23187 in endothelin-1 (3 × 10⁻⁸ M)-preconstricted pulmonary artery rings from broiler (n = 12) and Leghorn (n = 11) chickens. Data points are means ± SE. *P < 0.05 vs. Leghorn at same A23187 concentration.

Fig. 3. Relaxation responses to increasing concentrations of sodium nitroprusside in endothelin-1 (3 × 10⁻⁸ M)-preconstricted pulmonary artery rings from broiler (n = 14) and Leghorn (n = 29) chickens. Data points are means ± SE. *P < 0.05 vs. Leghorn at same sodium nitroprusside concentration.
rings up to an SNP concentration of $10^{-6}$ M. However, at the two highest concentrations of SNP ($10^{-5}$ and $10^{-4}$ M), relaxation responses in broiler were greater than in Leghorn PA rings and exceeded the original baseline (i.e., >100%). In addition, papaverine total relaxation responses also exceeded the original baseline and were greater in broiler than Leghorn PA rings (201.3 ± 7.8 vs. 171.2 ± 10.7%, respectively, $P < 0.05$).

Effects of L-NAME and L-arginine on ACh-induced relaxation responses in PA rings. Preincubation with L-NAME ($10^{-3.5}$ M) or L-arginine ($10^{-4}$ M) before the addition of ET-1 had no effect on baseline tension of rings from either of the chicken strains (data not shown). These two compounds, however, had significant effects on the relaxation responses of PA rings to ACh. Preincubation with L-NAME attenuated the concentration-dependent relaxation response to ACh in both broiler and Leghorn PA rings to a similar extent (Fig. 4). Thus, at all ACh concentrations used, relaxation responses continued to be smaller in broiler than in Leghorn PA rings after L-NAME. When PA rings were pretreated with L-arginine, ACh relaxation responses were enhanced in both chicken strains (Fig. 5).

In this case, however, the increase in relaxation response induced by L-arginine was significantly ($P < 0.05$) greater in the broiler PA rings. As a result, relaxation responses to ACh in broiler PA rings were no longer significantly different from those of Leghorn PA rings. In the presence of L-arginine, the greatest ACh concentration ($10^{-5}$ M) resulted in relaxations exceeding the original baseline (i.e., >100%) in both strains. Preincubation with D-arginine had no effect on the ring relaxation responses from either of the chicken strains (data not shown).

Effects of L-NAME and L-arginine on PA ring constriction responses to ET-1. When constriction responses to ET-1 ($10^{-7.5}$ M) are expressed as milligram of force per milligram of tissue (Fig. 6), preincubation with L-NAME induced a significantly greater constriction response to ET-1 in both broiler and Leghorn PA rings, suggesting EDNO is synthesized during ET-1-induced constriction. Although the increase caused by preincubation with L-NAME tended to be greater in Leghorn than in broiler PA rings compared with control, the difference in change was not significant. Similarly, preincubation with L-arginine induced a marginal reduction in constriction force in PA rings from both strains that was not significant. Pretreatment with D-arginine did not alter ET-1-induced responses in either chicken strain (data not shown).

DISCUSSION

The primary finding of this study is that PA rings from broiler chickens have reduced relaxation re-

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**Fig. 4.** Effect of structural analog of L-arginine (L-Arg), NG-nitro-L-arginine methyl ester (L-NAME) on relaxation responses to increasing concentrations of acetylcholine in endothelin-1 ($3 \times 10^{-8}$ M)-preconstricted pulmonary artery rings from broiler ($n = 18$ for both control and L-Arg) and Leghorn ($n = 10$ and 11 for control and L-Arg, respectively) chickens. Rings were preincubated for 45 min with $3 \times 10^{-4}$ M L-NAME in buffer solution or in buffer solution alone (controls) before endothelin-1 was added. Data points are means ± SE. *Means within acetylcholine concentration with no common letter are significantly different ($P < 0.05$).

**Fig. 5.** Effect of L-Arg on relaxation responses to increasing concentrations of acetylcholine in endothelin-1 ($3 \times 10^{-8}$ M)-preconstricted pulmonary artery rings from broiler ($n = 18$ for both control and L-Arg) and Leghorn ($n = 10$ and 11 for control and L-Arg, respectively) chickens. Rings were preincubated for 15 min with $10^{-4}$ M L-Arg in buffer solution or in buffer solution alone (controls) before endothelin-1 was added. Data points are means ± SE. *Means within acetylcholine concentration with no common letter are significantly different ($P < 0.05$).

**Fig. 6.** Constriction responses to $3 \times 10^{-8}$ M endothelin-1 in pulmonary artery rings from broiler and Leghorn chickens in absence (control; $n = 34$ and 18 for broiler and Leghorn rings, respectively) or presence of $10^{-4}$ M L-Arg (n = 17 and 10 for broiler and Leghorn rings, respectively) or $3 \times 10^{-4}$ M L-NAME ($n = 18$ and 12 for broiler and Leghorn rings, respectively). Bars represent means ± SE of constriction force normalized to ring tissue weight. *$P < 0.05$ vs. control.
responses to endothelium-dependent vasodilators compared with PA rings from Leghorn chickens. Endothelium-dependent relaxation responses to both ACh and the calcium ionophore A23187 were reduced in broiler vs. Leghorn PA rings. As both ACh and A23187 are EDNO vasodilators but A23187 is receptor independent, this suggests the reduced relaxation response to ACh in broiler PA rings was not determined at the endothelial cell cholinergic receptor level. In addition, endothelium-independent relaxations to the NO donor SNP were not different between broiler and Leghorn PA rings, except at the two highest SNP concentrations, which induced greater relaxation responses in broiler than in Leghorn PA rings. These data suggest that the cause for the reduced endothelium-dependent relaxations observed in broiler PA rings was not determined by a reduced responsiveness of the vascular smooth muscle to NO but likely located within the EDNO synthesis pathway. Furthermore, the enhanced relaxation response of broiler PA rings to levels different from Leghorn PA rings in the presence of L-arginine suggest that availability of substrate for NOS may be a key limiting factor for PA vasodilation in the broiler chicken. Plasma L-arginine concentrations were not different between the chicken strains, thus L-arginine may be limited to NOS at an intracellular pool within the endothelium. Finally, relaxation responses to ACh, SNP, and papaverine below the original baseline, which were greater with papaverine in broiler than in Leghorn PA rings, suggest that PA rings from broiler chickens have greater intrinsic tone than PA rings from Leghorns.

Meat-producing-type broiler chickens are highly susceptible to severe pulmonary hypertension, right heart failure, and ascites (16). Although this was originally reported in broiler chickens reared at high altitudes (11), it later became evident that the new strains of broiler chickens were highly susceptible to pulmonary hypertension regardless of the altitude at which they were reared (27). On the other hand, egg-producing-type Leghorn chickens have been shown to be highly resistant to severe pulmonary hypertension compared with broilers (23). It is believed that along with selection for rapid growth, anatomic and functional changes of the cardiopulmonary system have occurred, making broiler chickens highly susceptible to a ventilation-perfusion mismatch, hypoxemia, and, as a consequence, pulmonary hypertension. Indeed, rapid growth in chickens has been associated with lower oxygen diffusing capacity, hypoxemia, right ventricular hypertrophy, and ultimately with pulmonary hypertension and right-sided cardiac failure (17, 27, 28). In the present study broiler chickens grew 1.8 times faster than Leghorns, and, although pulmonary arterial pressure and blood oxygen pressure were not measured, a greater right-to-total ventricular weight ratio in broiler chickens suggests that broilers had a greater pulmonary arterial pressure than Leghorns (8). Recently, Wideman et al. (45) reported that after unilateral PA occlusion, similar responses, including an initial 100% increase in pulmonary vascular resistance and severe pulmonary hypertension, occurred in both broiler and unselected chickens. However, within minutes and via an apparent flow-dependent vasodilation, unselected chickens were able to reduce pulmonary arterial pressure and vascular resistance to levels not different from those before the occlusion, whereas broiler chickens were not (45, 48). This suggests that pulmonary vasodilation was responsible for reducing pulmonary hypertension in the unselected chickens and that vascular relaxation may be impaired in broilers.

It is now well established that the vascular endothelium actively participates in controlling vascular smooth muscle tone via the production and release of several vasoactive substances including EDNO (7). EDNO is produced in response to various vasodilator stimuli, including physiological and pharmacological agents, such as ACh and the calcium ionophore A23187 (26, 31). In chickens in particular, it was previously shown that ACh induces endothelium-dependent vasodilation of isolated aortae via production of EDNO (13). In other species, changes in EDNO synthesis have been reported to occur in several cardiovascular disorders (36). Recent studies using lung toxicity or hypoxia to increase pulmonary arterial pressure have shown that there are changes in EDNO production during pulmonary hypertension (2, 12, 32, 33, 35). However, whether these changes include an increase or a decrease in EDNO production is highly controversial. Results of the present study are in concordance with those of Adnot et al. (2) and Eddahibi et al. (12). Considering that broiler chickens are highly prone to pulmonary hypertension, their PA rings had very similar vasoactive characteristics compared with the isolated lung preparations from hypoxic rats (2, 12). Broilers and hypoxic rats exhibited reduced relaxation responses to endothelium-dependent vasodilators but not to endothelium-independent NO donors, and these reduced relaxation responses were restored by the addition of L-arginine, the substrate for NOS.

Accordingly, other reports have indicated that supplementing diets with L-arginine attenuates pulmonary hypertension in broilers (46, 47). These data support the concept that in the pulmonary vasculature of broiler chickens and hypoxic rats there is a deficiency in NOS substrate and a reduced synthesis of EDNO. Eddahibi et al. (12) reported that plasma levels of L-arginine in hypoxic rats were significantly lower than in control rats. In the present study, however, a difference in L-arginine plasma levels was not observed between broiler and Leghorn chickens, which may in part suggest that in broiler chickens, L-arginine may only be limited intracellularly. Furthermore, L-arginine may only be limited to NOS in a specific intracellular pool (9, 21, 39), and this may occur due to a reduced uptake of L-arginine by the endothelial cell (6). Alternatively, the ratio of arginine to NOS may be out of balance in the broiler PA endothelial cell, such that L-arginine degradation may be occurring predominantly via the arginase pathway (50).
As in the present study, Hasegawa et al. (13) reported that relaxation responses to ACh in chicken aortae were only partially blocked by L-NAME. This suggests that in chicken aortae and PA, ACh may induce endothelium-dependent relaxation via an additional factor other than EDNO. Alternatively the inhibitory effect of L-NAME on NOS may have different characteristics in chicken vs. mammalian vessels.

It has also been suggested that the intrinsic ability of NOS to synthesize NO may be impaired in hypoxia-induced pulmonary hypertension (35) and that availability of cofactors for NOS and not an intracellular deficiency of L-arginine may be the cause for a reduced synthesis of EDNO in diabetes (50). Although these are also plausible explanations for the reduced relaxation response observed in the broiler PA rings, the fact that L-NAME, a competitive inhibitor of NOS, decreased relaxation responses to a similar extent in both chickens strains and L-arginine enhanced relaxation responses to a greater extent in broiler than in Leghorn PA rings suggests that L-arginine is the main limiting factor. Contradictory reports indicating an increased synthesis of EDNO and an increased expression of NOS (14, 19, 32, 33) may also be explained by availability of L-arginine in an intracellular pool in different types of animals. This may be particularly important in animal species such as the chicken, in which de novo synthesis of L-arginine is not possible because of a deficiency of the enzyme carbamoyl phosphate synthase (40, 41). Thus, even in the event of an increased expression of NOS in pulmonary hypertension, limited availability of L-arginine may still cause the production of EDNO to be reduced.

In the present study, the fact that L-arginine significantly increased relaxation responses to ACh but did not significantly reduce the constriction induced by ET-1 appears paradoxical. Randall and Griffith (30) reported a similar phenomenon in that after L-NAME treatment, basal EDNO synthesis in preconstricted rabbit ear arteries was sensitive to L-arginine supplementation, whereas agonist-dependent EDNO synthesis was not. A possible explanation is that although ET-1 induces EDNO production via interaction with type B receptors in endothelial cells (18), EDNO may also be produced during ET-1 constriction via calcium entry into the endothelial cell induced by the smooth muscle contraction per se, and these events may involve different endothelial cell NOS isoforms with different sensitivities to L-arginine supplementation (10, 37). There is no doubt, however, that EDNO was produced during the ET-1-induced constriction, because L-NAME significantly increased the force of constriction in all PA rings.

The presence of relaxation responses extending below baseline in the present study suggests there is intrinsic tone in these arteries. A greater intrinsic tone in broiler than in Leghorn PA rings is suggested by the greater relaxation responses to SNP occurring beyond the baseline and is further corroborated by the greater total relaxation observed in broiler than in Leghorn PA rings when the nonspecific endothelium-independent vasodilator papaverine was used. Previously, intrinsic tone was reported in endothelium-denuded but not in endothelium-intact PA isolated from hypoxic rats (43, 44). In PA with intact endothelium, constriction responses were induced by increasing concentrations of L-NAME, suggesting there was basal release of EDNO (43, 44). In the present study, intrinsic tone was observed in endothelium-intact PA rings from both strains of chickens. The absence of a constriction response when L-NAME was added to the preparation before exposure to ET-1 suggests that these preparations did not have basal release of EDNO. Basal release of EDNO may be unique to PA in chickens because Hasegawa et al. (13) reported that L-NAME induced significant vasoconstriction in chicken aortae.

It could be argued that differences in intrinsic tone between the chicken strains in the present experiment were responsible for the differences in relaxation responses to endothelium-dependent vasodilators. If this had been the case, a greater intrinsic tone in broiler chickens should have resulted in greater relaxation responses to endothelium-dependent vasodilators such as those observed in response to the greatest concentrations of SNP. In addition, constriction responses to ET-1 were not significantly different between the strains, thus making percent relaxations comparable across strains and further indicating that differences in intrinsic tone were not responsible for the differences in endothelium-dependent relaxations. It is possible, however, that a greater intrinsic tone in the broiler PA rings was the result of a greater wall tension in broiler vs. Leghorn PA rings. According to Laplace law, a vessel of greater internal diameter develops greater wall tension than a smaller vessel subjected to the same pressure or, in this case, the same preload force. In the present experiments, PA ring weights along with body weights increased 1.8 times faster in broilers than in Leghorns. In addition, the internal diameter of the PA rings, although not measured, appeared to be larger in broiler than in Leghorn chickens. In other experiments, we found that absolute wall thickness is greater in larger pulmonary arteries and arterioles from fast-growing broilers compared with slow-growing broiler and Leghorn chickens (unpublished observations). Although a thicker vessel would increase diffusion time of substances across the vessel wall, relaxation responses to the endothelium-independent vasodilator SNP (10−10–10−6 M) in the present experiments were not different between chicken strains. As previously mentioned, constriction responses to ET-1 were not significantly different between the strains, suggesting that broiler PA rings, although larger, did not have a significantly increased constriction force. The greater intrinsic tone observed in PA rings from broilers may be responsible for the greater right-to-total ventricular weight ratio observed in broiler chickens, as it may be indicative of a greater pulmonary arterial pressure. Thus the cause of the increased susceptibility to pulmonary hypertension in broiler chickens may include a greater PA intrinsic tone and an impaired endothelium-dependent vasodilator capacity.
Primary pulmonary hypertension is a cardiovascular condition that affects young people and several animal species. Although primary pulmonary hypertension is by definition idiopathic in origin, recent studies have brought increasing knowledge about its pathophysiology and potential etiological factors. Among the difficulties in further elucidating the etiology and pathophysiology of this disease are the lack of primary pulmonary hypertension animal models. Current animal models consist of developing pulmonary hypertension secondary to lung damage or hypoxia. In these models, however, changes induced by hypertension and changes induced by the factors inducing hypertension are difficult to differentiate. Therefore, the cause of primary pulmonary hypertension can only be obscurely inferred from these models. In the present study we used the chicken as a model of primary pulmonary hypertension. Specific strains of chickens having different susceptibility to primary pulmonary hypertension are the basis of this model. This model represents a unique opportunity to obtain information before, during, and after the development of pulmonary hypertension in susceptible individuals (broiler chickens) and compare it with individuals with no propensity for the disease (Leghorn chickens). Our results support the presence of alterations in the production of vaso dilators by the vascular endothelium and an increased pulmonary arterial intrinsic tone in the pathogenesis of primary pulmonary hypertension. Although augmented intrinsic tone and endothelial dysfunction provide a plausible explanation for the clinical expression of the disease, many other mechanical and biochemical alterations encountered in patients with primary pulmonary hypertension have the potential for being etiological factors as well. With the knowledge generated using models of primary and secondary pulmonary hypertension, more effective preventive and therapeutic measures against this condition may be developed.

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