Heat shock protein 90-eNOS interactions mature with postnatal age in the pulmonary circulation of the piglet

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Aschner JL, Zeng H, Kaplowitz MR, Zhang Y, Slaughter JC, Fike CD. Heat shock protein 90-eNOS interactions mature with postnatal age in the pulmonary circulation of the piglet. Am J Physiol Lung Cell Mol Physiol 296: L555–L564, 2009. First published January 9, 2009; doi:10.1152/ajplung.90456.2008.—Binding of endothelial nitric oxide synthase (eNOS) to the chaperone protein, Hsp90, promotes coupled eNOS synthetic activity. Using resistance level pulmonary arteries (PRA) from 2-day-, 5- to 7-day-, and 12-day-old piglets, we tested the hypothesis that Hsp90-eNOS interactions are developmentally regulated in the early neonatal period. PRA were isolated for coimmunoprecipitation and immunoblot analyses or cannulated for continuous diameter measurements using the pressurized myography technique. NO inhibition caused less constriction in PRA from 2-day- compared with 5- to 7-day- and 12-day-old piglets. No age-related differences were found in dilation responses to an NO donor or in protein expression of Hsp90, phospho-eNOS (Ser1177), Akt, phospho-Akt, or caveolin-1. Compared with the older animals, PRA from 2-day-old piglets had higher total eNOS expression but displayed less binding of eNOS to Hsp90 and Akt. Hsp90 antagonism with radicicol induced greatest constriction in PRA from 12-day-old piglets. ACh stimulation caused dilation in PRA from 5- to 7-day- and 12-day-old but not 2-day-old animals, despite rapid and equivalent ACh-mediated eNOS phosphorylation (Ser1177) in all three age groups. Hsp90 inhibition abolished ACh-mediated dilation in PRA from the older piglets. ACh failed to stimulate Hsp90-eNOS binding in 2-day-old but induced a significant increase in Hsp90-eNOS coimmunoprecipitation in PRA from the older age groups, which was blocked by Hsp90 antagonism. We conclude that physical interactions between Hsp90 and eNOS mature over the first weeks of life, likely contributing to the postnatal fall in pulmonary vascular resistance and changes in agonist-induced pulmonary vascular responses characteristic of the early neonatal period.

Akt; development; nitric oxide

The early postnatal period is one of dramatic changes in the pulmonary circulation. Soon after birth, the neonatal pulmonary circulation undergoes vascular remodeling and a marked drop in pulmonary vascular resistance resulting in an 8- to 10-fold increase in pulmonary blood flow (1, 9, 12, 25, 40). The mechanisms responsible for postnatal changes in pulmonary vascular tone, reactivity, and architecture have long been of interest to pulmonary vascular biologists and neonatologists caring for infants with persistent pulmonary hypertension of the newborn (PPHN). Whereas pulmonary hypertension caused by genetic or environmental factors can occur at any postnatal age, the syndrome and pathology of PPHN is unique to the early newborn period. Delineating the mechanisms that underlie the normal postnatal changes in pulmonary vascular tone and reactivity could lead to novel therapies to treat infants who fail to undergo the normal postnatal circulatory transition.

A role for nitric oxide (NO) in normal perinatal circulatory transition is well-established. Prenatal inhibition of nitric oxide synthase (NOS) causes postnatal pulmonary hypertension (1, 11, 19). Differences in pulmonary vascular responses and NO expression between late fetal and early newborn life have been well-described (3, 26, 36, 38, 42, 47). Juvenile and adult animals demonstrate enhanced endothelium-dependent vasodilation compared with the fetus and newborn (2, 33). Changes in NO production and NOS expression and function have been implicated in these postnatal maturational changes in pulmonary vascular tone and reactivity (2, 10, 23, 24, 31, 33, 36, 38, 47, 49). This manuscript addresses a critical window of time in the first 2 wk of life when little is known about the regulation of NOS activity and the corresponding pulmonary vascular responses.

More recently, the complex regulation of NO production from endothelial NOS (eNOS) has been appreciated, involving a number of posttranslational modifications that are critical to eNOS synthetic activity. We (4) and others (28, 34, 46) have reported that the constitutively expressed chaperone protein, Hsp90, regulates vascular tone and NO production in the lung. The role of Hsp90-eNOS interactions in the early postnatal maturation of pulmonary vascular tone and reactivity remains unclear.

In this study, we tested the hypothesis that Hsp90-eNOS interactions are developmentally regulated in the early neonatal period. We further hypothesized that postnatal alterations in Hsp90 chaperone function has a functional impact on the regulation of pulmonary vascular tone over the first 2 wk of postnatal life. All of our studies in newborn piglets were performed with resistance level pulmonary arteries (PRA) because of their importance in the regulation of pulmonary vascular tone.

MATERIALS AND METHODS

Animals. Newborn piglets of three different age groups, 2 days (n = 22), 5–7 days (n = 17), and 12 days (n = 15), were preanesthetized with ketamine [30 mg/kg intramuscular (im)] and acepromazine (2 mg/kg im) followed by an intravenous injection of sodium pentobarbital (10 mg/kg) and heparin (1,000 IU/kg iv) and then...
exsanguinated. Heart and lungs were removed en block and stored in cold, oxygenated physiological bicarbonate solution (PBS) for up to 72 h before study. All experimental protocols were performed in adherence with the National Institutes of Health (NIH) guidelines for the use of experimental animals and approved by the Animal Care and Use Committee of Vanderbilt University Medical Center. The animal resource facility is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Isolation of PRA for measurement of pressurized diameter. Piglet PRA (80- to 250-μm diameter) were isolated, cannulated, and pressurized for continuous measurement of diameter according to our previously published methods (4, 5, 13, 15, 16, 20). Briefly, PRA measuring <250-μm diameter were dissected in oxygenated Krebs-Henseleit buffer with the following millimolar composition: 118 NaCl, 25 NaHCO3, 4.5 KCl, 1.2 MgCl2·6H2O, 1.2 KH2PO4, 11 dextrose, 2.5 CaCl2·2H2O, and 0.026 EDTA equilibrated with 21% O2, 5% CO2, and balance N2 to maintain pH 7.4. The arteries were measuring previously published methods (4, 5, 13, 15, 16, 20). Briefly, PRA were pressurized for continuous measurement of diameter according to our PRA study protocol. After a 30-min equilibration period with a stable diameter, vascular smooth muscle and endothelial function were assessed by contraction to KCl (50 mM) or the thromboxane receptor antagonist, radicicol (20 nM), on baseline tone and ACh-mediated vasodilation. The linear mixed effects model generalizes the commonly used linear regression model by including additional terms such as a random intercept and random slope to control for correlation and thus provide valid inference. As each PRA was exposed to cumulative concentrations of the drug, we included a random intercept and random slope to account for the correlation arising from taking repeated observations on the same PRA (Figs. 1, 2, 6, 7, and 9).

Nonparametric ANOVA (Kruskal-Wallis or the Wilcoxon signed-rank test) was used to examine concentration-dependent and age-dependent changes in PRA diameter to L-NAME, SNAP, and ACh. The linear mixed effects model generalizes the commonly used linear regression model by including additional terms such as a random intercept and random slope to control for correlation and thus provide valid inference. As each PRA was exposed to cumulative concentrations of the drug, we included a random intercept and random slope to account for the correlation arising from taking repeated observations on the same PRA (Figs. 1, 2, 6, 7, and 9).

Nonparametric ANOVA (Kruskal-Wallis or the Wilcoxon signed-rank test) was used to examine the association between protein expression and postnatal age (Figs. 3 and 7). Kruskal-Wallis followed by the Mann-Whitney U test was used to analyze the effect of a single exposure to radicicol on baseline tone (Fig. 5). Coimmunoprecipitation studies were analyzed by one-way ANOVA with Fisher protected least significant differences test (Fig. 4) or by paired t-tests (Fig. 8).

Results are reported as P values with P < 0.05 indicating statistical significance.

RESULTS

To determine the NO-dependent contribution to basal tone at each age group, pressurized PRA were exposed to increasing concentrations (1–100 μM) of the NOS inhibitor, L-NAME. As shown in Fig. 1, PRA from 2-day-old piglets display minimal responses to NOS inhibition. At each L-NAME concentration, PRA from the two older age groups demonstrate a greater constriction compared with the PRA response in 2-day-old animals.

One possible explanation for the failure of L-NAME to cause constriction in PRA from 2-day-old piglets is diminished downstream sensitivity to NO. However, as shown in Fig. 2, SNAP-mediated functional responses are not only intact in PRA from 2-day-old piglets, but also more robust at intermediate concentrations of SNAP.

Protein expressions of eNOS, phospho-eNOS (Ser1177), Hsp90, Akt, phospho-Akt, and caveolin-1 were examined in PRA homogenates from the three age groups. Representative
Western blots are shown in Fig. 3A; Fig. 3B shows the summary densitometry data for each protein relative to actin. No significant age-related differences were noted in the expressions of phospho-eNOS (Ser1177), Hsp90, Akt, phospho-Akt, and caveolin-1. Expression of total eNOS was higher in PRA from 2-day-old piglets than in PRA from the older two age groups.

Despite more total eNOS protein in PRA from 2-day-old piglets (Figs. 3 and 4), communoprecipitation assays demonstrated less binding of eNOS to Hsp90 in PRA from 2-day-old piglets compared with the two older age groups (Fig. 4A). Similarly, there was less Akt bound to eNOS in the 2-day-old vs. older piglets (Fig. 4B).

To demonstrate the functional importance of Hsp90 chaperone function, we examined the effect of the Hsp90 antagonist, radicicol, on basal tone in PRA from the three age groups. As shown in Fig. 5, radicicol induces the greatest constriction in PRA from the 12-day-old piglets.

Agonist-mediated responses in the three age groups are shown in Fig. 6. ACh, a NO-dependant agonist, causes dilation from basal tone in PRA from 5- to 7-day- and 12-day-old piglets but does not cause dilation in PRA from 2-day-old piglets.

The representative Western blots in Fig. 7A and summary densitometry graphs in Fig. 7B demonstrate a rapid and similar increase in the expression of phospho-eNOS [phospho-eNOS (Ser1177)] following stimulation with ACh (0.1 μM) for 10, 30, or 60 s in all three age groups. The magnitude and time course of phospho-eNOS expression following ACh stimulation did not differ among the three age groups. ACh stimulation had no effect on the expression of total eNOS protein (Fig. 7C).

Figure 8 demonstrates that ACh stimulates the binding of eNOS to Hsp90 with a near doubling of the amount of Hsp90 that coimmunoprecipitates with eNOS in 5- to 7-day- and 12-day-old PRA (Fig. 8, B and C). In contrast, ACh fails to enhance the coimmunoprecipitation of Hsp90 and eNOS in PRA from the 2-day-old piglets (Fig. 8A). Furthermore, the Hsp90 antagonist, geldanamycin, prevents the increase in association between Hsp90 and eNOS stimulated by ACh in the two older age groups.

Lastly, we evaluated the effect of Hsp90 antagonism on vascular responses to ACh. We chose to use radicicol rather than geldanamycin for these functional studies as geldanamycin has been shown to cause redox cycling. Redox cycling may have relatively less impact on physical associations measured by communoprecipitation. Figure 9A shows that radicicol has no significant effect on ACh responses in 2-day-old piglets. In comparison, radicicol abolishes dilation in PRA from the 5- to 7-day-old animals. This effect is nearly identical to our previously published findings in PRA from 12-day-old piglets (13) where we showed that both radicicol and geldanamycin abolish ACh-mediated dilation.

**DISCUSSION**

Pulmonary circulatory transition at birth is thought to be regulated, at least in part, by release of NO from eNOS (1, 11, 18, 19, 27). Maturational changes in the regulation of pulmonary vascular tone by NO have been described in a number of species, including rat (10, 26, 36, 49), pig (24), lamb (23, 38), baboon (41), and human (30, 31). Prior publications comparing fetal and postnatal expression of eNOS have demonstrated an increase in NO expression during gestation that is maximal shortly before or at the time of birth (26, 36) with a subsequent decline postnatally to the low levels observed in adults (36, 47, 49). These studies have been limited by an experimental approach that used whole lung homogenates, cultured endothelial cells, or large conduit pulmonary arteries; the contribution of eNOS from PRA has not been previously addressed.

Novel findings in this study are that physical interactions between Hsp90 and eNOS mature over the first 2 wk of life in piglets.

**Fig. 1.** Effect of postnatal age on resistance level pulmonary artery (PRA) responses to N^ω^-nitro-l-arginine methyl ester (l-NAME). PRA from piglets aged 2 days (n = 16 PRA from 9 piglets), 5–7 days (n = 7 PRA from 7 piglets), and 12 days (n = 11 PRA from 7 piglets) were exposed to increasing concentrations (Conc) of l-NAME. At each concentration, l-NAME caused a greater constriction in PRA from 5- to 7-day- and 12-day-old piglets than in 2-day-old piglets. *P < 0.001, different from 2-day-old.

**Fig. 2.** Effect of postnatal age on PRA responses to S-nitroso-N-acetyl penicillamine (SNAP). PRA from piglets aged 2 days (n = 8 PRA from 5 piglets), 5–7 days (n = 10 PRA from 10 piglets), and 12 days (n = 8 PRA from 6 piglets) were exposed to increasing concentrations of the NO donor, SNAP. SNAP induced a concentration-dependent dilation in PRA from all 3 age groups. Dilation responses to SNAP were similar in PRA from the 5- to 7-day-old; ##P = 0.001, entire concentration-response curve different from 5- to 7-day-old and 12-day-old. ##P = 0.001, entire concentration-response curve different from 5- to 7-day- and 12-day-old.
Moreover, we provide evidence that these postnatal changes in Hsp90-eNOS interactions have important functional impact. Specifically, our findings are the first to show that maturation in Hsp90-eNOS interactions underlie, at least in part, the postnatal changes in both basal and agonist-induced pulmonary vascular responses that occur over the first days and weeks of life.

Our data demonstrate that endogenous NO does not play a major role in control of resting tone in 2-day-old piglets but suggest an important role of basal NO release in resting tone of older newborns (Fig. 1). This finding is similar to those of other investigators who have examined the impact of NOS inhibition in lambs (22, 44) and piglets (33) at comparable ages. One possible explanation for the failure of L-NAME to cause constriction in PRA from the 2-day-old animals is diminished downstream sensitivity to NO. However, as shown in Fig. 2, PRA from 2-day-old piglets are capable of robust dilation to the NO donor, SNAP. These findings indicate that downstream sensitivity to NO is not impaired and cannot explain the reduced responses of the younger animals to NOS inhibition. Indeed, at intermediate concentrations, dilation to SNAP was greatest in the 2-day-old PRA. This finding is consistent with that of Berkenbosch et al. (7), who found that an NO donor elicited a more robust dilation in the isolated perfused lungs of 4-day-old compared with 15-day-old piglets. Likewise, sodium nitroprusside induced greater relaxation in large pulmonary artery rings from 2-day- compared with 1-mo-old lambs (37).

The mechanisms underlying these findings might be related to increased expression or greater activity of soluble guanylate cyclase (sGC) in newborns compared with more mature animals as reported in carotid and cerebral arteries in the ovine species (48). An increase in pulmonary sGC mRNA, protein levels, and activity in the perinatal period has also been reported in the rat (6, 8). In contrast, Moreno et al. (35) found an age-related increase in dilation to bubbled NO in conduit arteries between 1-day- and 2-wk-old piglets that correlated...
with increased sGC protein content in the large pulmonary arteries of the older piglets.

We also explored the possibility that age-related changes in NOS expression underlie the functional differences in vascular responses in the three age groups. We found increased, not decreased, total protein expression of eNOS in PRA from the 2-day-old piglets (Figs. 3 and 4) without significant differences in phospho-eNOS (Ser1177), Akt, phospho-Akt, or caveolin-1, a protein when bound to eNOS is thought to inhibit NOS activity (Fig. 3). Prior published studies have compared eNOS expression in fetal, 1-day-old, and juvenile or adult animals, reporting lower eNOS expression in the juvenile and adult animals relative to the fetus and 1-day-old newborn (3, 26, 38). North et al. (36) found eNOS is highest in the late gestation fetus and is similar in 1- and 5-day-old rats. Kawai et al. (26) found no differences in pulmonary eNOS protein or mRNA expression in rats between days 1 and 16 of life, and Chicoine et al. (10) reported similar total eNOS protein in the lungs of 1- and 2-wk-old rats. Sheffield et al. (43) found no significant differences in eNOS expression in postmortem human newborn lung tissue with advancing gestational age or severity of chronic lung disease. Although differences in expression of other NOS isoforms could contribute to maturational changes in vascular responses in the lung, other investigators have reported no differences in iNOS (10) or nNOS (10, 36) expression in whole lung immunoblots over the first 2 wk of life.

Hsp90 is a chaperone protein that is critical to the regulation of eNOS activity (4, 39). Hsp90 acts as a protein scaffold for expression in fetal, 1-day-old, and juvenile or adult animals, reporting lower eNOS expression in the juvenile and adult animals relative to the fetus and 1-day-old newborn (3, 26, 38). North et al. (36) found eNOS is highest in the late gestation fetus and is similar in 1- and 5-day-old rats. Kawai et al. (26) found no differences in pulmonary eNOS protein or mRNA expression in rats between days 1 and 16 of life, and Chicoine et al. (10) reported similar total eNOS protein in the lungs of 1- and 2-wk-old rats. Sheffield et al. (43) found no significant differences in eNOS expression in postmortem human newborn lung tissue with advancing gestational age or severity of chronic lung disease. Although differences in expression of other NOS isoforms could contribute to maturational changes in vascular responses in the lung, other investigators have reported no differences in iNOS (10) or nNOS (10, 36) expression in whole lung immunoblots over the first 2 wk of life.

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**Fig. 4.** Coimmunoprecipitation of eNOS with Hsp90 and Akt. PRA protein homogenates were immunoprecipitated (IP) with anti-eNOS antibody, and the resultant immunoprecipitate was subjected to immunoblot (IB) analysis using antibodies against eNOS, Hsp90, and Akt. A demonstrates that there is less binding of eNOS to Hsp90 in PRA of 2-day-old piglets compared with PRA from piglets aged 5–7 or 12 days. Similarly, less Akt is bound to eNOS in PRA of 2-day-old vs. older piglets (B). *P < 0.05, different from 2-day-old. n = 3 different piglets in each age group.

**Fig. 5.** Effect of radicicol on basal diameter. Radicicol had no impact on baseline lumen diameter in 2-day-old piglet PRA (n = 9 PRA from 9 piglets) and caused a significantly greater constriction in PRA from the 12-day-old piglets (n = 5 PRA from 5 piglets) compared with the 5- to 7-day-old (n = 12 PRA from 12 piglets) and 2-day-old piglets. *P < 0.01, different from 2-day-old.

**Fig. 6.** Effect of postnatal age on PRA responses to ACh. ACh caused dilation of PRA from 5- to 7-day-old (n = 12 PRA from 12 piglets) and 12-day-old piglets (n = 7 PRA from 7 piglets) but did not induce dilation in PRA from 2-day-old piglets (n = 10 PRA from 5 piglets). *P < 0.002, different from 2-day-old.
a multiprotein complex that facilitates phosphorylation by Akt/PKB of eNOS on Ser1177 and activation of the enzyme. Other investigators found that basal amounts of phosphorylated eNOS on Ser1177 (34) and basal Hsp90 protein levels (46) differed between pulmonary artery endothelial cells of fetal and 4-wk-old lambs. However, data on expression of phospho-eNOS and the NOS regulatory proteins, Hsp90 and Akt, in the early postnatal period have, to our knowledge, not been re-

![Fig. 7. Time course of ACh-stimulated phosphorylation of eNOS on Ser1177. In all 3 age groups, ACh (0.1 μM) stimulation for 10, 30, or 60 s increased the amount of eNOS that is phosphorylated on Ser1177 (A and B). No significant differences were detected in the magnitude or time course of eNOS phosphorylation among the 3 age groups. ACh stimulation had no effect on total eNOS at any time point in any of the 3 age groups (C) (n = 5 piglets in each age group). Top: representative Western blots in the 3 age groups. Middle: summary densitometry data showing the ratio of phospho-eNOS relative to actin following stimulation with ACh for 10–60 s in each age group. Bottom: summary densitometry data showing the ratio of total eNOS relative to actin following stimulation with ACh for 10–60 s in each age group. *P = 0.0002, different from time 0; #P = 0.0001, different from time 0; ^P = 0.0129, different from time 0.](http://ajplung.physiology.org/)

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Hsp90/eNOS co-immunoprecipitation
IP: eNOS; IB: eNOS and Hsp90

A
Representative immunoblots and summary data from 2-day old piglets

B
Representative immunoblots and summary data from 5-7-day old piglets

C
Representative immunoblots and summary data from 12-day old piglets

Factors that impact eNOS uncoupling include limited availability of substrate, arginine (14, 17), or cofactor, tetrahydrobiopterin (34), or lack of adequate Hsp90-eNOS binding (4, 28, 46). All of these factors could be contributing to the reduced eNOS-dependent vascular responses in the 2-day-old piglets.

New findings from this study indicate that interactions between proteins important for regulating eNOS activity change between days 2 and 12 of postnatal life. Our data indicate that at least one explanation for the poor response to the NOS antagonist in the 2-day-old PRA is limited Hsp90-eNOS binding (Fig. 4). Figure 5 showing markedly reduced alterations in basal tone on inhibition of Hsp90 chaperone function with
raticicol had no significant effect on ACh responses (5- to 7-day-old piglets). Pretreatment of PRA from 2-day-old piglets with radicicol abolished ACh-mediated dilation. *P < 0.001, different from untreated. n = 8 PRA from 4 2-day-old piglets; n = 7 PRA from 7 5- to 7-day-old piglets.

Fig. 9. A and B: effects of radicicol on ACh responses in PRA from 2-day- and 5- to 7-day-old piglets. Pretreatment of PRA from 2-day-old piglets with radicicol had no significant effect on ACh responses (P = 0.50). In PRA from 5- to 7-day-old piglets, radicicol abolished ACh-mediated dilation. *P < 0.001, different from untreated. n = 8 PRA from 4 2-day-old piglets; n = 7 PRA from 7 5- to 7-day-old piglets.

raticicol further supports this concept. Under conditions of limited Hsp90-eNOS binding, phospho-eNOS produces superoxide anion, reflecting uncoupling between electron flow, phosphorylation, and NO production. Moreover, our findings suggest that between days 2 and 12 of life in the piglet, Hsp90-eNOS binding matures so that more eNOS is bound to Hsp90 (Figs. 4A and 5). These findings support a growing body of literature that points to the importance of Hsp90 chaperone function in assuring the correct multiprotein stoichiometry of complexes between Hsp90 and eNOS (44, 50). Our data expand on these observations by showing that this postnatal improvement in NOS-dependent dilation is due, at least in part, to a change in the capacity for Hsp90 binding to eNOS in response to agonist stimulation with ACh (Figs. 8 and 9). The ability of ACh to induce enhanced Hsp90-eNOS binding is deficient in 2-day-old piglets (Fig. 8A), and this correlates with the poor ACh-mediated dilation responses in this age group (Fig. 6) despite intact agonist-induced increases in phospho-eNOS (Fig. 7). Thus, similar to our findings in unstimulated PRA and reports by others (21, 45), we show that there is no direct correlation between the amount of agonist-stimulated phospho-eNOS (Ser1177) and Hsp90-eNOS binding or functional dilation.

ACh responses are due, at least in part, to alterations in muscarinic receptor expression or distribution within the pulmonary arterial tree. It is also possible that our findings are unique to ACh and that stimulation with other eNOS agonists, such as bradykinin or A-23187, would yield a different result.

The enhanced agonist-stimulated Hsp90-eNOS association with postnatal maturation in the first 2 wk of life that we report differs from the findings of Mata-Greenwood et al. (34), who failed to detect a developmental increase in Hsp90-eNOS association on stimulation of cultured ovine pulmonary artery endothelial cells with A-23187. Important differences between the study by Mata-Greenwood et al. (34) and our study include the use of a single agonist, ACh, to stimulate eNOS. In contrast to the functional dilations observed in the 12-day-olds may reflect more basal activation of eNOS in pressurized 12-day-old PRA than in pressurized vessels from the younger age groups or may reflect interactions between Hsp90 and a client protein other than eNOS that influences basal tone. In contrast to the functional studies with radicicol, the responses to L-NAME and SNAP primarily reflect inhibition or stimulation, respectively, of the NO-cGMP pathway.

Our data also shed light on the impact of postnatal maturation on agonist-induced eNOS biochemistry and functional responses. As well as regulating basal tone, maturational changes in Hsp90-eNOS interactions could have a significant impact on the ability of the pulmonary vasculature to respond to endogenous or exogenous stimuli that regulate vascular tone via the NO-cGMP signaling pathway. Other investigators have similarly shown that responses to the NOS-dependent agonist, ACh, increase over the first days to months of postnatal life (2, 32, 33, 44, 50). Our data expand on these observations by showing that this postnatal improvement in NOS-dependent dilation is due, at least in part, to a change in the capacity for Hsp90 binding to eNOS in response to agonist stimulation with ACh. Other limitations prevent our recapitulating these findings in intact, pressurized PRA from the three age groups. Other limitations of our study include the use of a single agonist, ACh, to stimulate eNOS. It is possible that age-related changes in ACh responses are due, at least in part, to alterations in muscarinic receptor expression or distribution within the pulmonary arterial tree. It is also possible that our findings are unique to ACh and that stimulation with other eNOS agonists, such as bradykinin or A-23187, would yield a different result.

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Taken together, our findings show that, compared with 5- to 7-day- and 12-day-old piglets, 2-day-old piglets have limited ability to form complex multiprotein interactions between Hsp90, eNOS, and Akt. Our findings also suggest that this limitation in regulatory protein interactions correlates with a reduced role for endogenous NO in the regulation of pulmonary vascular tone in the immediate postnatal period. Moreover, the contribution of NO to pulmonary vascular tone and reactivity increases in the first 2 wk of life in association with enhanced Hsp90-eNOS binding and concomitant with the normal fall in pulmonary vascular resistance, which occurs over the first weeks of life. Thus biophysical interactions between Hsp90 and eNOS, which mature over the first weeks of life, likely contribute to the postnatal fall in pulmonary vascular resistance and changes in pulmonary vascular responses characteristic of the early neonatal period.

GRANTS

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