Natriuretic peptide C receptor in the developing sheep lung: role in perinatal transition

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BACKGROUND: At birth, the release of surfactant from alveolar type II cells (ATIIs) is stimulated by increased activity of the beta-adrenergic/adenylyl cyclase/cyclic 3′-5′ adenosine monophosphate-signaling cascade. Atrial natriuretic peptide (ANP) stimulates surfactant secretion through natriuretic peptide receptor A (NPR-A). ANP inhibits adenylyl cyclase activity through its binding to NPR-C. We wished to further understand the role of the NPR-C in perinatal transition.

METHODS: We studied ATII expression of NPR-C in fetal and newborn sheep using immunohistochemistry, and surfactant secretion in isolated ATIIs by measuring ^{3}[H] choline release into the media.

RESULTS: ANP induced surfactant secretion, and, at higher doses, it inhibits the stimulatory effect of the secretagogue terbutaline. ATII NPR-C expression decreased significantly after birth. Premature delivery also markedly decreased ANP and NPR-C in ATIIs. Co-incubation of terbutaline (10^{-4} M) with ANP (10^{-6} M) significantly decreased ^{3}[H] choline release from isolated newborn ATII cells when compared with terbutaline alone; this inhibitory effect was mimicked by the specific NPR-C agonist, C-ANP (10^{-10} M).

CONCLUSION: ANP may act as an important epithelial-derived inhibitor of surfactant release in the fetal lung, and downregulation of ANP and NPR-C following birth may sensitize ATII cells to the effects of circulating catecholamines, thus facilitating surfactant secretion.

Respiratory distress syndrome (RDS) is associated with significant morbidity and mortality in premature neonates. Full maturation of the pulmonary epithelium occurs relatively late in gestation; therefore, infants delivered prematurely are less capable of establishing and maintaining a normal gas exchange interface. Central to this problem is the deficient level of pulmonary surfactant released from alveolar type II epithelial cells (ATIIs) into the alveolar space, resulting in elevated surface tension and the clinical condition of neonatal RDS. The clinical use of maternal antenatal steroids and surfactant replacement therapy has improved the prevention and treatment of RDS; however, the cellular mechanisms responsible for the control of surfactant release remain less well understood. Regulation of ATII maturation and surfactant release are influenced in large part by late-term elevations of intracellular cyclic 3′-5′ adenosine monophosphate (cAMP) generated by increased activity of the adenylyl cyclase pathway (1).

Atrial natriuretic peptide (ANP) is a member of the natriuretic peptide (NP) family that includes B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The NP receptors (NPRs) consist of the guanylyl cyclase (GC)-linked receptors, NPR-A and NPR-B, and the non-GC-linked receptor NPR-C. NPR-A is specific for ANP and BNP, whereas NPR-B is bound preferentially by CNP. Binding of the NPs to the extracellular domain of NPR-A and NPR-B results in activation of intracellular GC, catalyzing the conversion of guanosine triphosphate (GTP) into the second messenger guanosine 3′, 5'-cyclic monophosphate (cGMP) (2). Distinct from NPR-A and NPR-B is the non-GC-linked receptor, termed NPR-C, which has roughly equal affinity for all three NPs. Although NPR-C possesses an extracellular ligand-binding region similar to that of the other NPRs, it contains a comparatively short intracellular domain of 37 amino acids that is responsible for adenylate cyclase (AC) inhibition through the stimulation of G_i-1 and G_i-2 (3,4).

We previously reported that both ANP and its primary receptor, NPR-A, are highly expressed in ATIIs in the fetal lung before birth, but that shortly after birth this expression disappears (5). ANP at 10^{-10} M stimulated surfactant secretion in fetal and newborn sheep ATIIs. NPR-Aactivates the cGMP second messenger pathway to stimulate surfactant secretion. However, in a variety of cell types *in vitro*, ANP is also capable of inhibiting adenylyl cyclase by binding to NPR-C. A large body of evidence exists on the role of the NP system in cardiovascular homeostasis in health and disease states. BNP has been utilized both in the diagnosis and treatment of congestive heart failure and patent ductus arteriosus in preterm infants (6–8). There is very limited information on the NP

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system and its functional significance in the respiratory system. Although the intracellular mechanism by which ANP inhibits AC has been elucidated, the physiologic relevance of this interaction in the respiratory system has been lacking.

In this set of experiments, we aim to study the ontogeny of the natriuretic peptide C receptor and its effect on surfactant secretion in the perinatal period during the transition from placental to pulmonary gas exchange.

METHODS

Isolation of ATIIs from Fetal and Newborn Lambs

All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the State University of New York at Buffalo. The technique utilized for the isolation and culture of ATIIs has been characterized in detail previously by our laboratory and is outlined briefly (5). For fetal lambs (136 days of gestation, full term is 145 days), pregnant ewes were anesthetized by an intravenous injection of pentobarbital sodium (2.3 mg/kg) and placed under isoflurane (1.5%) general anesthesia. Following delivery by cesarean section, the fetal chest cavity was exposed by a midline sternotomy, and the animal was killed by rapid exanguination through a cardiac puncture. Warmed Hanks Balanced Salts Solution (HBSS) (Sigma, St Louis, MO) was instilled into the pulmonary vasculature through the right atrium, and a catheter fitted with a stopcock was fed into the left main bronchus and tied firmly in place. "Newborn" lambs (3 days following spontaneous term vaginal delivery) were killed by an intravenous injection of 5 ml pentobarbital sodium (390 mg/ml) (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). The remainder of the surgical protocol for newborn lambs was the same as that described above for fetal lambs.

ATIIs were isolated from left lungs by a combination of differential centrifugation followed by panning on plastic culture plates coated with sheep IgG. Following lavage with saline, a barium sulfate solution (Barium Sulfate 15 mg (Sigma) in 100 ml normal saline) was instilled and incubated at 37 °C for 15 min. The barium solution was aspirated, the lung was lavaged, and then instilled with a protease solution (DNAase type I 2 mg (Sigma), Elastase (5.1 u/mgP) 3.0 ml) (Worthington Biochemical, Lakewood, NJ), JMEM 200 ml; 37 °C) for 30–35 min at 37 °C. The protease incubation was stopped by the instillation of 100 ml of Minimal Essential Medium Joklik Modification with L-Glutamine (JMEM) ((Sigma), $NaHCO_3$ 2 g/l, HEPES 2.4 g/l, H_2O 1 liter; pH = 7.6) JMEM with 10 ml of fetal bovine serum. The digested lung was minced with scissors, filtered through a series of gauze mesh (150, 40, and 15 µm), and spun at 2,400 rpm for 6 min at 4 °C to pellet the cells. The cell pellet was re-suspended in 40 ml JMEM and 5 ml aliquots were centrifuged on a Percoll gradient (12 ml of Percoll 1.04 and 2 ml of Percoll 1.08). The cell band between the 1.04 and 1.08 layers was drawn off and re-suspended in 40 ml with JMEM. Following initial cell counts and viability assessments, the cell suspension was purified further by differential adherence on culture dishes (100 × 15 mm) coated with sheep IgG. Sheep immunoglobulin-coated plates were prepared using a method adapted from Dobbs *et al.* (9).

Measurement of Surfactant Secretion from Primary Cultures of ATIIs Isolated From Fetal and Newborn Lambs

Following the ATII isolation procedure, the cell pellet was re-suspended in warm Dulbecco's Modified Eagle Medium (Life Technologies, Grand Island, NY; $NaHCO_3$ 3.7 mg/l, H_2O 1 liter; pH = 7.4) with antibiotics/antimycotics (Life Technologies; Penicillin G 10,000 units/ml, streptomycin 10,000 µg/ml, Amphotericin B 25 µg/ml, and 10% charcoal-stripped fetal bovine serum to a final concentration of 1.5 million cells/ml). To assess surfactant release, 1 µci ^{3}[H] choline chloride/ml (Amersham, Arlington Heights, IL) was added to the cell suspension. Two milliliters (3 million cells) of the cell suspension was added to each well of a six-well plate and incubated for 16–18 h in a 5% CO_2 incubator at 37 °C. The following day, cells were removed from the incubator, the various agents were added, and the plates were returned to the incubator for 3 h. Compounds tested in the surfactant secretion assay included ANP (human, 1–28), 10^{-10} M and 10^{-6} M; DesQ,S,G,L,G,ANP (4–23)-NH_2 (C-ANP (4–23)), 10^{-10} to 10^{-6} M (Peptides International, Louisville, KY); terbutaline sulfate (Brethine, Switzerland, East Hanover, NJ)), 10^{-5} M, (Novartis, Switzerland, East Hanover, NJ)). PTX 0.5 µg/ml (Calbiochem, San Diego, CA) was added as a 2-h pre-incubation in some experiments. Lipid extractions on the media and cell scrapings were performed according to a previously described method (10), and counts for ^{3}[H] were obtained as CPM using a Wallac 1409 liquid scintillation counter. Data were expressed as percent surfactant release using the following calculation: [counts per minute in the extracted media/(counts per minute in the extracted media+counts per minute in the extracted cells)] × 100. Surfactant secretion data were analyzed by one-way analysis of variance followed by Bonferonni's *post hoc* test for multiple comparisons using STATA software (College Station, TX).

Immunohistochemistry for NPR-C

The protocol for immunohistochemistry of fetal and newborn lamb lung sections has been described in detail (11) by our laboratory previously. Briefly, fetal lambs (136 days gestation) and newborn lambs (3 days following term vaginal delivery) were killed, and lung tissue was obtained using the same method described above for ATII cell isolations. The right lower lobe was removed at the level of the main stem bronchus and instilled with formalin for 24 h. Following fixation, lung tissue blocks were embedded in paraffin and immunostained for NPR-C (goat anti-human NPR-C, biotinylated: 1:100, overnight incubation at room temperature, Santa Cruz Biotech, Santa Cruz, CA). Antibody binding was visualized using SigmaFast DAB (3,3′-diaminobenzidine tetrahydrochloride) and H_2O_2 (Sigma), followed by counterstaining with Mayer's hematoxylin (Sigma).

To assess the possible regulation of NP family expression in ATIIs prenatally and postnatally, we delivered preterm lambs at 125 days' gestation and ventilated them for 24 h. We then performed IHC as detailed above. Immunostaining for PreproANP was performed using Rabbit polyclonal anti-human PreproANP 1:1,500 incubated overnight at 4 °C (Peninsula Lab, Belmont, CA).

RESULTS

Immunohistochemistry for NPR-C in Fetal and Newborn Lamb Lung

At 100 days of gestation, the cuboidal epithelial cells lining the developing distal airspaces were positive for NPR-C. Positive NPR-C staining was clearly visible within the morphologically appearing ATIIs and Clara cells from lambs at 136 days of gestation, but it was not present in ATIIs and was reduced in Clara cells from 3-day-old to 4-week-old lambs (**Figure 1**).

Effect of ANP on Surfactant Release from Fetal and Newborn Lamb ATIIs

^{3}[H] PC release increased both in fetal and newborn ATIIs on co-incubation with ANP. Maximal stimulation occurred at 10^{-10} M, and further increase in dose caused less surfactant secretion (**Figure 2**).

Effect of ANP on Terbutaline-Stimulated Surfactant Release from Fetal and Newborn Lamb ATIIs

Following the addition of terbutaline alone, terbutaline stimulated surfactant release as expected. ^{3}[H] PC release from

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near-term fetal ATIIs increased by 210% above baseline and by 95% above baseline for term newborn ATIIs (Figure 3). The ability of terbutaline to simulate ^{3}[H] PC release was not significantly altered by the addition of 10^{-10} M ANP; however, the addition of 10^{-6} M ANP significantly reduced terbutaline-stimulated PC release by greater than 70% from newborn ATIIs and trended toward a decrease in fetal ATIIs (Figure 3).

Effect of the Specific NPR-C Ligand C-ANP (4–23) Upon Basal and Terbutaline-Stimulated Surfactant Release from Newborn Lamb ATIIs

In order to study this effect of ANP on inhibiting an effect mediated via the beta-2 adrenergic pathway (terbutaline-stimulated surfactant secretion), we used an ANP analog that is specific for only the NPR-C, rather than ANP itself, which binds to both NPR-A and NPR-C (Figure 4). The addition of 10^{-10} M C-ANP reduced terbutaline-stimulated ^{3}[H] PC release by more than 90%. When given alone, C-ANP at doses ranging from 10^{-10} M to 10^{-8} M had no effect on ^{3}[H] PC release.

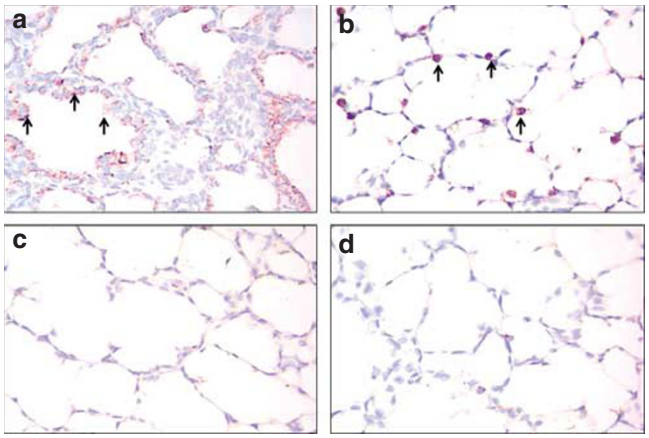

Figure 1. Immunostaining for natriuretic peptide receptor (NPR)-C in distal lung epithelium of fetal and newborn lambs (all ×200). Distal lung epithelial cells are positive for NPR-C at 100 days (**a**). Intense staining for NPR-C is present in alveolar type II cells (ATIIs) by 136 days (**b**). At 3 days (**c**) and 4 week (**d**) post gestation, NPR-C staining in ATIIs is almost undetectable.

Effect of Pertussis Toxin Inactivation of G_i on C-ANP (4–23)-Mediated Inhibition of Terbutaline-Stimulated Surfactant Release from Newborn Lamb ATIIs

The effect of G_i blockade on NPR-C-mediated inhibition of terbutaline-stimulated ^{3}[H] PC release was examined by the co-administration of C-ANP with pertussis toxin (PTX; Figure 5). The ability of C-ANP to inhibit terbutaline-stimulated ^{3}[H] PC release was blocked in the presence of PTX, confirming that the C-ANP-induced inhibition of terbutaline-stimulated surfactant secretion is mediated via the known G_i-mediated beta-adrenergic pathway used by terbutaline. PTX alone did not significantly alter terbutaline-stimulated ^{3}[H] PC release.

Effect of Preterm Delivery on NP Family Expression

To determine whether the marked decrease in NP expression postnatally was truly developmentally regulated or due to birth itself, we studied NP system expression in fetal and prematurely delivered lambs. We found that birth, even at a very premature gestation (125 days, 145 days is term) equivalent to respiratory functional maturity comparable to 26–28 weeks' gestation in the human, is associated with the same marked decrease in NPR-C (Figure 6) and prepro-ANP

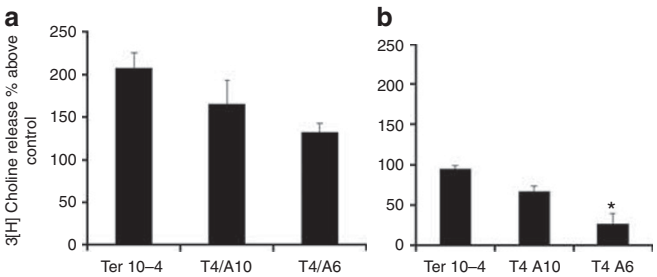

Figure 3. Effect of atrial natriuretic peptide (ANP) on terbutaline-stimulated phosphatidylcholine secretion from fetal (**a**) and newborn (**b**) lamb alveolar type II cells. Cells were plated and labeled overnight with 3[H] choline (1 μci/ml) followed by a 3 h stimulation with terbutaline (100 μM) with or without ANP (100 pM and 1 μM). Values are the mean ± SEM of four experiments run in duplicate. Asterisk denotes significant difference from terbutaline alone ($P < 0.001$ by analysis of variance).

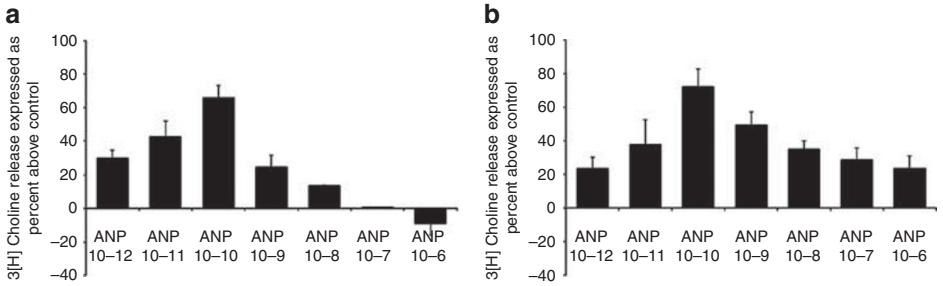

Figure 2. Effect of atrial natriuretic peptide (ANP) on phosphatidylcholine secretion from fetal (**a**) and newborn (**b**) lamb alveolar type II cells—dose–response curve. Cells were plated and labeled overnight with 3[H] choline (1 μci/ml) followed by a 3 h stimulation with varying concentrations of ANP. Values are the mean ± SEM of four experiments run in duplicate.

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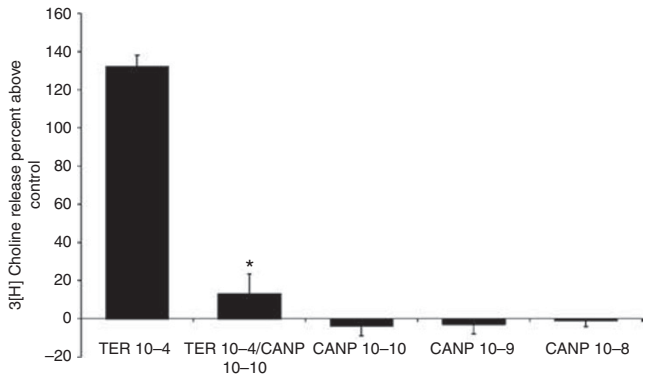

Figure 4. Effect of the NPR-C-specific ligand C-ANP (4–23) on terbutaline-stimulated phosphatidylcholine secretion from newborn lamb ATIIs. Cells were plated and labeled overnight with 3[H] choline (1 μci/ml) followed by a 3 h stimulation with terbutaline (100 μM) with or without C-ANP (4–23) (100 pM) and C-ANP (4–23) alone (100 pM–10 nM). Values are the mean ± SEM of three experiments run in duplicate. Asterisk denotes significant difference from terbutaline alone ($P < 0.001$ by analysis of variance).

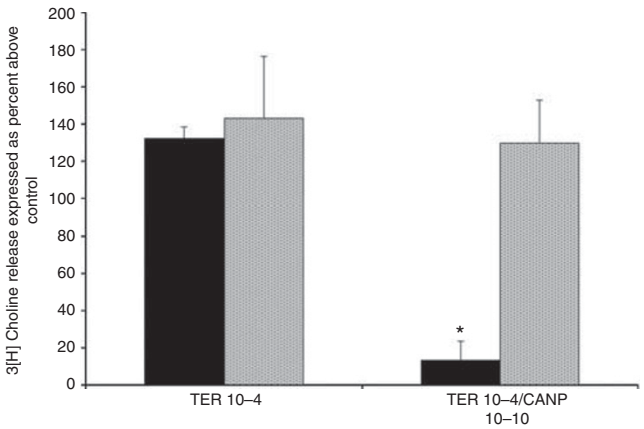

Figure 5. Effect of pertussis toxin (PTX) pretreatment on C-ANP (4–23)-mediated inhibition of terbutaline-stimulated 3[H] choline release from newborn lamb alveolar type II cells. Cells were plated and labeled overnight with 3[H] choline (1 μci/ml). Cells were stimulated for 3 h with 100 μM terbutaline with (solid black bars) or without (patterned gray bars) 3 h pretreatment with PTX (0.5 μg/ml). Values are the mean ± SEM of three experiments run in duplicate. Asterisk denotes significant difference from terbutaline alone ($P < 0.001$ by analysis of variance).

expression (**Figure 7**) that we saw in term newborn sheep, suggesting that birth itself is the stimulus for the change in expression.

DISCUSSION

During the late-term fetal and immediate newborn period, adequate control of pulmonary surfactant release is essential for establishing and maintaining a functional gas exchange interface after birth. Previous studies have shown that the late-term fetus and newborn are exposed to elevated levels of circulating ANP and that the perinatal lung contains large numbers of binding sites for the natriuretic peptides (12,13). Using an *in vitro* assay, we had previously shown that ANP at doses ranging from 1 to 100 pM stimulated, in a dose-dependent manner, the release of PC from ATIIs isolated from both fetal and newborn lambs. The stimulation of PC secretion by ANP results from ANP binding to NPR-A, resulting in the production of the intracellular second messenger cGMP (5).

We have shown that ANP at higher (μM) doses inhibits terbutaline-stimulated PC release from fetal and newborn ATIIs. This finding is in agreement with previous studies involving a wide array of cell systems demonstrating that ANP acts as an inhibitor of exocytosis by decreasing intracellular levels of cAMP. The inhibitory effect of ANP on cell secretion has been reported in other cell systems including progesterone secretion from Leydig cells (14), thyroglobulin secretion from the thyroid (15), and catecholamine release from pheochromocytoma cells (16,17). Several lines of evidence have shown that ANP-mediated decreases in cAMP are the result of adenylyl cyclase inhibition by NPR-C. Analysis of the cytoplasmic domain of NPR-C has revealed a 17-amino-acid sequence capable of activating the inhibitory G proteins ($G_{i\alpha1}$ and $G_{i\alpha2}$), resulting in the inhibition of adenylyl cyclase (3). More recent studies have demonstrated that

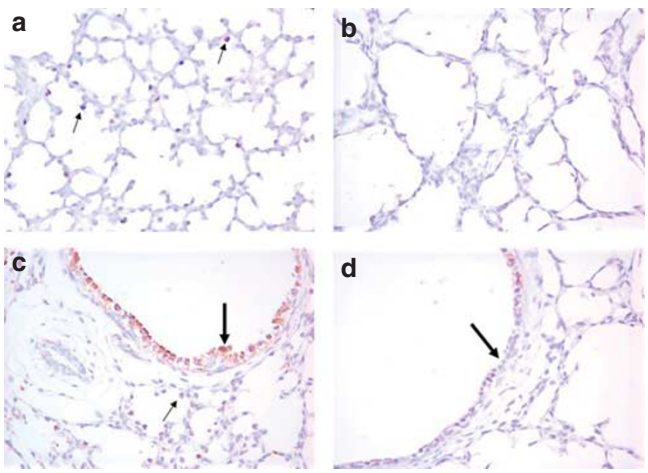

Figure 6. Immunostaining for natriuretic peptide receptor (NPR)-C in 125-day fetal lambs and 125-day lambs following delivery and ventilation for 24 h. Non-ventilated fetal lambs (**a**) and (**c**) demonstrate positive NPR-C staining in alveolar type II cells (small arrows), Clara cells, and ciliated bronchial epithelial cells (large arrow) (×200). Following 24 h of ventilation (**b**, **d**), NPR-C staining is absent in epithelium of the upper and lower airways (×200).

NPR-C mutants that lack a specific region of the cytoplasmic domain are no longer capable of AC inhibition and that exogenous administration of the deleted peptide sequence restores the inhibitory activity of NPR-C (4,18,19).

Our study is the first to demonstrate that ANP has a biphasic effect on surfactant secretion, i.e., at lower doses, it has the effect of surfactant secretion, previously shown to be via the NPR-A receptor as this effect was blocked by Rp-8-Br-PET-cGMPS, a PKG inhibitor. At higher doses, ANP is less stimulatory, and in fact these higher doses also block the

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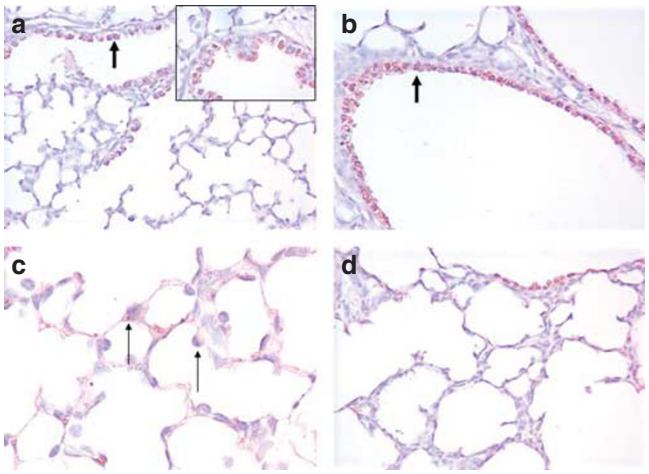

Figure 7. Immunostaining for prepro-atrial natriuretic peptide (ANP) in 125-day fetal lambs and 125-day lambs following delivery and ventilation for 24 h. Non-ventilated fetal lambs (**a**, **c**) demonstrate positive staining in Clara cells (large arrow) and weaker staining in alveolar type II cells (small arrow) (inset ×400). Following 24 h of ventilation (**b**, **d**), pre–pro-ANP staining is still present in Clara cells (large arrow) and absent in type II cells (×200).

well-known effect of terbutaline to stimulate surfactant secretion. The NPR-C-specific ligand, C-ANP (4–23), significantly inhibited terbutaline-stimulated PC release from ATIIs, and this effect is blocked by pretreatment of cells with PTX, demonstrating that C-ANP blocks the known effect of terbutaline-induced secretion through the beta adrenergic receptor. We conclude that activation of NPR-C on ATIIs inhibits beta-adrenergic-stimulated surfactant secretion through a G-protein-coupled mechanism. A previous study using ATIIs isolated from adult rats demonstrated that ANP at μM doses resulted in a similar dose-dependent G-protein-mediated blockade of terbutaline-stimulated PC release (20). In that study, the authors speculated that the inhibitory effect of ANP was due to NPR-C activation, based primarily on their understanding of that receptor's effects on various G-protein-mediated pathways. Our data further support this hypothesis by demonstrating that in newborn ATIIs, the effect of micromolar concentrations of ANP is mimicked by the NPR-C-specific ligand, C-ANP (4–23). C-ANP (4–23) is a ring-deleted analog of ANP with a high specificity for NPR-C. Previous reports have stated some concern over the specificity of C-ANP (4–23) at higher concentrations (21). In order to address those concerns, we utilized concentrations of C-ANP (4–23) well below that known to activate NPR-A, reported to be in the high nanomolar range. In our study, C-ANP (4–23) at a dose of 100 pM was highly effective in blocking terbutaline-stimulated PC release without an effect on the basal release of surfactant. Furthermore, we found that C-ANP (4–23) at concentrations ranging from 100 pM to 10 nM did not alter the basal release of PC. This finding indicates that C-ANP (4–23) at the concentration given in our study did not possess activity consistent with NPR-A activation.

Although previous studies have shown elevated levels of plasma ANP in the fetus and the presence of ANP and NPR-C in the fetal lung, this is the first study to demonstrate a possible physiological role for the ANP/NPR-C axis in normal pulmonary development. The *in vitro* effects of NPR-C activation taken together with the high levels of NPR-C and ANP protein expression found only in fetal ATIIs suggest a possible mechanism through which elevated levels of ANP and NPR-C may act to regulate cAMP-mediated surfactant release during the fetal period. We hypothesize that the downregulation of NPR-C at birth may act to sensitize ATIIs to the effects of circulating catecholamines, enhancing the release of surfactant in the newborn period.

The significance of intracellular levels of cAMP within fetal ATIIs extends beyond its role in regulating surfactant release. It is well recognized that increases in circulating catecholamines and intracellular elevations in cAMP levels during late gestation contribute to the differentiation and maturation of ATIIs. *In vitro* and *in vivo*, cAMP has been shown to play an integral role in ATII maturation by increasing the synthesis and release of phospholipids and surfactant-associated proteins (22,23). It has been known for some time that culture of ATIIs results in alterations in cell phenotype, characterized by progressive loss of lamellar bodies and downregulation of surfactant protein production. Ultimately, cultured ATIIs assume a phenotype consistent with alveolar type I cells. Studies by Gonzales *et al* have shown that the addition of cAMP to ATII cultures is capable of maintaining a mature ATII phenotype (24). Taken together, our findings indicate that decreases in NPR-C expression may contribute to the rise in intracellular cAMP and the establishment of a mature ATII phenotype.

Using specific antibodies, we investigated the developmental expression of NPR-C in the lung. The very high homology of the NP among species permitted the use of antibodies raised against NP from species other than sheep. The mature 28-amino-acid form of ovine ANP is identical to human ANP (25). In addition, the human ANP (1–28) peptide used in our *in vitro* studies has been shown previously to possess potent physiologic activity in sheep whole-animal studies (26). We selected 100 days of gestation as the earliest time point for our immunohistochemical studies because during this period the lamb ATIIs become identifiable by the appearance of lamellar bodies (27). The cell-specific expression of NPR-C was identical to that of the GC-linked receptors. However, NPR-C expression appeared in the fetal lung earlier and was prominent in distal lung epithelium, airway smooth muscle, and in chondrocytes at 100 days of gestation. We did not examine time points earlier than 100 days of gestation and therefore do not know the onset of NPR-C expression in the lung. Currently, no information exists concerning pulmonary NPR-C protein or gene expression during early fetal development. Similar to the other NPRs, NPR-C expression in pulmonary epithelium declined sharply following birth and was absent in ATIIs by 3 days after birth. Because term in the fetal lamb is ~145 days and our latest time point examined was at 136 days, we cannot rule out the possibility that NPR-C downregulation occurs in response to stimuli during late

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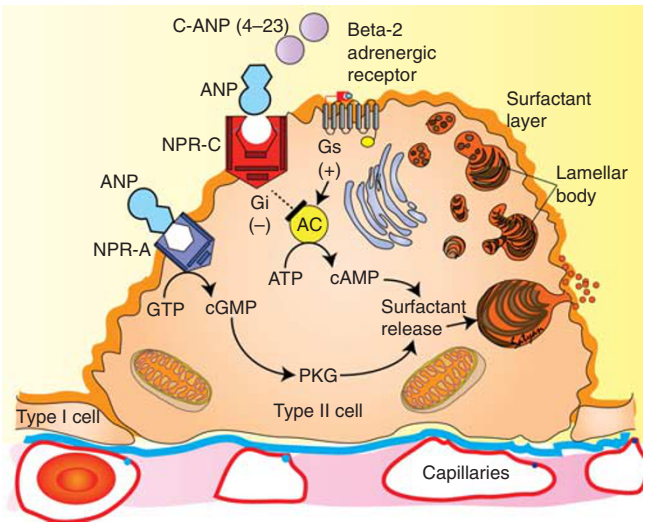

Figure 8. Proposed working model of the effect of ANP and NPR-C on surfactant secretion in the perinatal period. ANP at lower doses binds to the NPR-A receptor and stimulates surfactant release. At higher doses, ANP also binds to the NPR-C receptor and inhibits beta-2 agonist stimulation of surfactant release. Based on this model and the dramatic changes in the expression of ANP and NPR-C in the type II cell before and after birth, the natriuretic peptide system appears to play a critical role in neonatal transition.

gestation such as elevations in glucocorticoids. It has been shown that in the near-term fetal lamb, antenatal steroid treatment significantly reduces pulmonary NPR-C mRNA expression (28). This may be one mechanism that explains the positive effect of antenatal steroids to reduce the risk of RDS.

In summary, our findings have led us to construct a working model (**Figure 8**) describing the role of ANP in surfactant release from fetal and newborn ATIIs. This model is complex and includes a dual role for ANP in the control of surfactant release from ATIIs. First, our data indicate that ANP at low doses is capable of stimulating surfactant release through the activation of its GC-linked receptor, NPR-A. Second, ANP at higher doses significantly inhibits terbutaline-stimulated surfactant release, an effect we believe to be mediated through the activation of NPR-C. In addition to our mechanistic data, our immunohistochemical staining demonstrates elevated levels of NPR-A, NPR-C, and ANP synthesis in ATII cells of late gestational age fetal lambs. Taken together, these data have led us to speculate that locally elevated levels of ANP in the fetal lung stimulate predominately NPR-C, resulting in the inhibition of beta agonist-stimulated surfactant release until birth. We speculate that at birth, even premature birth, the downregulation of NPR-C and of ANP synthesis by ATII sensitizes ATII cells to the effects of circulating beta agonists, thus enhancing surfactant release and lung liquid clearance in the immediate newborn period.

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Disclosure: The authors declare no conflict of interest.

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