Influence of glucocorticoids, neuregulin-1β, and sex on surfactant phospholipid secretion from type II cells

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In this study, it has been shown that glucocorticoids enhance both β-adrenergic receptor (β-AR) activity and (−)-isoproterenol-induced secretion of surfactant. This effect is known to be more apparent in cells derived from female fetuses, a characteristic that has been attributed to sex-linked differences in the fibroblasts. In the current study, it has been shown that dexamethasone enhances both β-adrenergic receptor (β-AR) activity (1.3- to 1.6-fold increase) and (−)-isoproterenol-induced secretion of surfactant (1.8- to 1.9-fold increase) in type II cells. However, fibroblast-conditioned media (FCM), prepared in the presence of dexamethasone, generates a much greater response to (−)-isoproterenol (3.1- to 3.8-fold increase). Furthermore, each of these effects is more pronounced if both cell types are female-derived. It is hypothesized that the enhanced response to glucocorticoids is the result of a synergistic effect between the steroid and a component of FCM. Neuregulin-1β (NRG1β), which is elevated in FCM generated in the presence of dexamethasone, enhances both β-AR activity in fetal type II pneumocytes resulting in a corresponding increase in (−)-isoproterenol-induced secretion of surfactant phospholipids from those cells. The extent of this effect was examined in the presence and absence of fibroblast-conditioned media (FCM) and in cells derived from fetuses of each sex. The peptide FPF was initially identified as a component of FCM (32, 35, 37) and is known to mediate the stimulatory effect of glucocorticoids on surfactant phospholipid synthesis. Given that NRG1β is now recognized as a likely component of FPF (10), it was examined for its ability to stimulate surfactant secretion and to mimic the effect of glucocorticoids on β-AR activity, β-AR gene expression, and (−)-isoproterenol-induced secretion of surfactant

MATERIALS AND METHODS

Animals. Nineteen-day pregnant rats of the Wistar strain of Rattus norvegicus were used for all experiments. The full gestation period of these animals is 22 days, and fetuses were delivered ~3 days premature. Animals were supplied by the Animal Resource Centre (Murdoch, Australia). The mating protocol involves caging male and female rats overnight, followed by vaginal smearing the next morning. If sperm are detected, then conception is considered to have occurred. This is accurate to within 8 h of actual conception and is considered to be day 0. Sex of the fetuses was determined by differences in the external genitalia, which can be recognized as early as day 17 of gestation. The small urogenital orifice and the genital swellings are larger in males than in females. Also, the distance between the rectum and the urogenital sinus is greater in male rat fetuses (27). All experiments complied with National Health and Medical Research Council guidelines, and were approved by the Murdoch University Animal Ethics Committee (Permit no. R2314/10).

Materials. All reagents were supplied by Sigma Aldrich (St. Louis, MO) unless otherwise stated. Eagle’s minimal essential medium (MEM) and newborn bovine serum were obtained from Thermo Fisher Scientific (Waltham, MA). Radiolabeled compounds were supplied by GE Healthcare (Little Chalfont, UK). Sterilized water was obtained from a Milli-Q system (Millipore, Billerica, MA). Recombinant human NRG1β and recombinant rat leptin (PeproTech, Rocky Hill, NJ) were used for all experiments. The full gestation period of these animals is 22 days, and fetuses were delivered ~3 days premature. Animals were supplied by the Animal Resource Centre (Murdoch, Australia). The mating protocol involves caging male and female rats overnight, followed by vaginal smearing the next morning. If sperm are detected, then conception is considered to have occurred. This is accurate to within 8 h of actual conception and is considered to be day 0. Sex of the fetuses was determined by differences in the external genitalia, which can be recognized as early as day 17 of gestation. The small urogenital orifice and the genital swellings are larger in males than in females. Also, the distance between the rectum and the urogenital sinus is greater in male rat fetuses (27). All experiments complied with National Health and Medical Research Council guidelines, and were approved by the Murdoch University Animal Ethics Committee (Permit no. R2314/10).

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Hill, NJ) were supplied as lyophilized powders and reconstituted in Milli-Q water for use. The SV total RNA isolation (catalog no. Z3100) and the OneStep RT-PCR (catalog no. 210212) kits used for RT-PCR reactions were purchased from Promega (Madison, WI) and Qiagen Sciences (Germantown, MD), respectively.

Preparation of materials for cell culture. Newborn calf serum (NBS) was incubated with sterile, acid-washed charcoal to remove endogenous steroids as previously described (34). Charcoal-treated serum was sterilized using a 0.22-μm filter and stored at −20°C before use. Rat immunoglobulin G was either purified using the method of Hudson and Hay (19) or obtained commercially (Sigma Aldrich). Eagle’s MEM was reconstituted as specified by the manufacturer and supplemented with 0.2% NaHCO₃. This mixture was then adjusted to pH 7.4 before l-glutamine, penicillin G, and streptomycin sulfate being added to final concentrations of 2.6 mM, 100 IU/ml, and 135 μM, respectively. Medium was sterilized by filtration through a 0.22-μm filter, and amphotericin was added to a final concentration of 3.2–3.6 μg/ml. To yield complete medium, charcoal-treated NBS was added to a final concentration of 10% serum.

Isolation of fibroblasts and type II pneumocytes. Nineteen-day pregnant rats were killed by asphyxiation with CO₂, and fetuses were treated NBCS was added to a final concentration of 10% serum.

Fetal lungs were removed, minced, and incubated with collagenase (0.05 IU/ml) for 20 min in a 37°C shaking water bath, as previously described (9, 34). After filtration through two layers of sterile gauze, the cells were centrifuged at 20 g for 2 min, and the pellet was resuspended in serum-free medium. This suspension was plated (6 lungs/plate) on 6-cm-diameter culture plates (Corning Life Sciences, Lowell, MA). These plates were incubated for 30 min at 37°C in a humidified incubator (5% CO₂, 95% air) (Thermo Fisher Scientific) to allow adhesion of fibroblasts. Following incubation, nonadhering cells were removed by gentle swirling, and medium was replaced with serum-containing medium. The nonadhering cells were used to isolate type II pneumocytes, according to the method of Dobbs et al. (12). Type II pneumocyte cultures were given an initial media change 24 h after plating and thereafter at 2-day intervals. After three days in culture the plates were nearly confluent and consisted of predominantly differentiated type II cells, each containing numerous lamellar bodies and being capable of both synthesis (34) and secretion (2, 9) of surfactant phosphatidylcholine.

Preparation of FCM. The medium of nearly confluent fibroblast cultures was changed to serum-free MEM with and without 100 nM dexamethasone (final concentration). After a 24-h incubation, the media were collected and stored in sterilized containers at −20°C. At the time of use, the conditioned media were thawed, heated at 60°C for 1 h [to destroy inhibitory factors known to be present in FCM (36)], filter sterilized using a 0.22-μm Millipore GS filter, and diluted 1:4 with serum-free MEM supplemented with the appropriate additive (either 100 nM dexamethasone or propylene glycol). This was applied to type II pneumocyte cultures for 24 h to test its effect on β-AR activity and (−)-isoproterenol-induced secretion of surfactant phospholipids.

Determination of β-AR activity in type II pneumocytes. Cultured type II pneumocyte cultures were incubated for a further 24 h with serum-free MEM containing 100 nM dexamethasone or the equivalent volume of propylene glycol (vehicle), with FCM (prepared as described above), or with 50 ng/ml NRG1β. At the end of this incubation, the cells were evaluated for β-AR activity using a technique previously described by Lefkowitz et al. (22). After removal of the FCM from the type II cells were washed with 170 mM Tris·HCl buffer, pH 7.6, containing the protease inhibitor, phenylmethylsulfonyl fluoride.
ride (10 μM) and air-dried for 2 h. The cells were incubated with 5 nM of the β-AR radioligand [3H]DHA solubilized in the same buffer. Nonspecific binding was estimated in the presence of 10 μM (−)-propranolol. After 150 min incubation, the type II pneumocytes were washed three times with Tris-HCl buffer, wiped on Whatman GF/A glass-fiber filter papers, and transferred to counting vials before the determination of radioactivity. The specific receptor binding, expressed as fmol [3H]DHA bound/μg DNA, was determined by subtracting the amount bound in the presence of (−)-propranolol (non-specific binding) from the amount bound in its absence (total binding).

Determination of surfactant phospholipid secretion. The medium from confluent type II cell cultures was removed and replaced with serum-free MEM containing [methyl-3H]choline chloride (1 μCi/ml). Following incubation for a further 24 h, the cultures were washed three times with 3 ml of balanced salts solution to remove unincorporated choline and any detached cells before adding 1.7 ml of serum-free MEM. The cells were allowed to equilibrate at 37°C in a CO2 incubator for 1 h before removing two plates to determine the media and cellular content of radiolabeled phospholipids as previously described (4, 9). To the remaining plates (two for each treatment) was added 17 μl of MilliQ water as vehicle (control) or the indicated concentrations of NRG1β or leptin. The media and cellular content of radiolabeled phospholipids was again determined after 3 h incubation of the cells with the peptides. The amount of [3H]phospholipids secreted by cells during the 3-h incubation period was then calculated as follows:

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\% \text{ secretion} = \frac{\text{dpm in media phospholipids} - \text{dpm in cellular phospholipids}}{100} 
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Confluent type II pneumocyte cultures were incubated for a further 24 h with serum-free MEM or FCM, each containing 100 nM dexamethasone or the equivalent volume of propylene glycol (vehicle), or with 50 ng/ml NRG1β. During this time the cells were simultaneously exposed to 1 μCi/ml [methyl-3H]choline chloride to label surfactant phospholipids. After 24 h exposure the cells were washed three times with BSS to remove unincorporated [methyl-3H]choline chloride, the media was replaced with 1.7 ml of serum-free MEM, and the cells were equilibrated for a further 1 h before the addition of 1 μM (−)-isoproterenol or an equivalent volume of vehicle (20 μg/ml of ascorbic acid in 0.01 M HCl). The amount of radiolabeled surfactant phospholipids secreted after a further 3 h was determined as described above.

RT-PCR quantification. The RT-PCR primers for GAPDH mRNA quantification were designed to include a splice junction between the products of mRNA and genomic DNA based on the size of the products. Because the β-AR gene has no introns or splice junctions (14, 21), it was necessary to confirm that the RNA extraction procedure, which includes the use of DNase, is sufficient to completely remove any contaminating genomic DNA. When the RNA extracts were treated with RNase before the β-AR RT-PCR and the products subjected to agarose gel electrophoresis, the band corresponding to the β-AR product was completely lost indicating no genomic DNA contamination. Each of the primer sequences chosen was checked for melting temperature using Primer3 software, lack of cross reactivity with other sequences (using BLAST software), and neither primer dimer formation nor interference with reference gene primers in a multiplex reaction. The forward and reverse primer sequences chosen for GAPDH (NCBI Reference Sequence: NM_017008) and β-AR (NCBI Reference Sequence: NM_012492.2) mRNA assays as follows: GAPDH (207 bp) forward 5′-agacagcgcactctctgtgtgt-3′ and reverse 5′-cttgccctggtgagtagcat-3′; β-AR (152 bp) forward 5′-ggagactgtgctgtgactt-3′ and reverse 5′-ctcggatttgtctcctct-3′. A similar rationale was used to design the TaqMan probes except that their melting temperatures were ~10°C higher than either of the forward and reverse primers. The probes for GAPDH and β-AR were designed with an Iowa Black quencher at the 3′-end and FAM (green) and HEX (yellow) covalently attached at the 5′-end, respectively, to allow the two products to be distinguished in multiplex RT-PCR reactions. The sequence of these probes is as follows: GAPDH probe, 5′-FAM-cctggtgacagttggccttc-(IABkFQ)-3′; probe β-AR, dye HEX, sequence 5′-(IABkFQ)-ctctcttctgtgcctgtgactt-3′.

Total RNA from the cell pellet was extracted using the protocol for the SV Total RNA Isolation System (Promega). The RNA extracts were stored at −70°C until quantification, which involved the use of an Agilent 2100 Bioanalyzer with an Agilent RNA 6000 Pico kit (Agilent Technologies, Santa Clara, CA). The extracts were diluted such that an aliquot containing 5 ng of mRNA was added to each 25-μl RT-PCR reaction. To measure relative gene expression, QIAGEN OneStep RT-PCR was undertaken using a QIAGEN Rotor-Gene Q6000 (QIAGEN, Hilden, Germany). For each RNA a test reaction was set up in an ultraviolet-sterilized, RNase-free PCR tube (Corning, Tewksbury, MA) containing forward and reverse primers as well as the TaqMan probes for the reference gene (GAPDH) and the gene of interest (β-AR). To optimize the RT-PCR conditions, a test was conducted by varying the annealing temperature between 55 and 59°C, and it was established that 57°C provided the best specificity and the highest yield of product. Thus, the reaction mixtures were incubated at 50°C for 30 min followed by denaturation at 95°C for 15 min. This was followed by 40 cycles using the following parameters: 94°C for 30 s, 57°C for 30 s, and 72°C for 60 s. The reactions were held at 10°C higher than either of the 

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\text{Fold increase above control} = \frac{\text{dpm in media phospholipids} - \text{dpm in cellular phospholipids}}{100} 
\]
RESULTS AND DISCUSSION

Effect of dexamethasone and FCM on β-AR levels and (-)-isoproterenol-induced secretion of surfactant phospholipids in fetal type II cells. β-AR activity was elevated from 0.17 ± 0.03 to 0.26 ± 0.03 and from 0.22 ± 0.04 to 0.29 ± 0.03 fmol/μg DNA in male- and female-derived fetal type II cells, respectively, if the cells were pretreated for 24 h with 100 nM dexamethasone (Fig. 1A). However, when the type II cells were exposed to media that had been conditioned by fibroblasts from the same gender in the presence of dexamethasone, there was an even greater increase in the β-AR activity to 0.30 ± 0.04 (P < 0.05) and 0.41 ± 0.03 (P < 0.01) fmol/μg DNA in male- and female-derived cells, respectively. Thus, the greatest increase in β-AR activity in type II cells (1.9-fold increase) was observed when female-derived type II pneumocytes were exposed to FCM from female-derived fibroblasts. Previous observations that glucocorticoids enhance the rate of transcription of the β-AR gene (1, 24, 26) were shown to be consistent with the finding that the β-AR gene contains a glucocorticoid response element (8).

Given that the β-agonist (-)-isoproterenol is known to enhance surfactant phospholipid secretion from fetal rat type II cells through its interaction with β-ARs (5, 13), any change in the β-AR activity would be expected to also result in an elevated rate of surfactant secretion in response to (-)-isoproterenol. When type II cells derived from either male or female fetuses were incubated with dexamethasone or FCM prepared in the presence of the steroid, there was no significant change in the rate of basal surfactant phospholipid secretion (Fig. 1B). In contrast, when

Fig. 4. Effect of NRG1β on β-AR and (-)-isoproterenol-induced secretion of surfactant phospholipids. Cultured rat type II pneumocytes derived from either male or female fetuses were exposed to either 50 ng/ml NRG1β (gray bars) or sterilized water (white bars). After 24 h the cells were washed three times to remove NRG1β and then analyzed for the cellular β-AR activity (A) and the ability of 1 μM (-)-isoproterenol (white hatched and gray hatched bars) or an equivalent volume of ascorbic acid solution (vehicle, white bars and gray bars) to stimulate surfactant phospholipid secretion (B). The results represent means ± SE of either 3 (B) or 5 (A) separate experiments. Any result that is significantly different from its corresponding control is indicated with asterisks (*P < 0.05 and **P < 0.01).
type II cells from male and female fetuses were grown in the absence of dexmamethasone and exposed to (-)-isoproterenol, there was a marked elevation in the rate of secretion of surfactant (1.7- and 1.9-fold, respectively; \( P < 0.01 \)). This enhancement by the \( \beta \)-agonist was significantly greater if the male- and female-derived type II cells were previously exposed to dexamethasone (2.2- and 2.4-fold, respectively; \( P < 0.001 \)). When the male- and female-derived type II pneumocytes were exposed to media conditioned by fibroblasts in the presence of dexamethasone, a treatment that produced the highest level of \( \beta \)-AR activity in the type II cells, there was an even greater response to (-)-isoproterenol (3.1- and 3.8-fold, respectively; \( P < 0.001 \)). Figure 2 demonstrates that there is a very strong correlation \(( r^2 = 0.903 \)) between the \( \beta \)-AR activity in type II cells and the extent to which (-)-isoproterenol stimulates secretion of surfactant phospholipids. Moreover, the data show that the responsiveness is greater under all conditions when the type II pneumocytes are derived from female fetuses.

**Influence of NRG1\( \beta \) on surfactant phospholipid secretion.** Because the (-)-isoproterenol-induced secretion of surfactant phospholipids is greatest in type II pneumocytes exposed to both dexamethasone and FCM, it suggests that a component of the conditioned media, such as FPF (35), may influence the responsiveness of these cells to the glucocorticoid. In a recent publication we proposed that one of the likely components of FPF is NRG1\( \beta \), an agent known to profoundly enhance the rate of surfactant phospholipid synthesis (10). When directly applied to type II cells, NRG1\( \beta \) also significantly increases surfactant phospholipid secretion in a time- and concentration-dependent manner (Fig. 3, A and B). When fetal type II pneumocytes were incubated for 3 h with NRG1\( \beta \), in the concentration range of 20–100 ng/ml, the surfactant phospholipid secretion increased by more than twofold at each concentration. The maximal stimulation was apparent at 50 ng/ml NRG1\( \beta \) (2.4-fold; \( P < 0.05 \)).

When cultured type II cells were exposed to 50 ng/ml NRG1\( \beta \) for 24 h there was also a marked increase in the activity of the \( \beta \)-AR \(( P < 0.05 \)) (Fig. 4A). This effect of the peptide was equally apparent in cells derived from either male or female fetuses. Given this stimulatory effect of NRG1\( \beta \) on \( \beta \)-AR activity, it is not surprising that, whereas (-)-isoproterenol only marginally increased the rate of secretion in control cells, it induced a 2.5-fold greater increase \(( P < 0.01 \)) if the cells had been previously exposed to 50 ng/ml NRG1\( \beta \) (Fig. 4B). It should be noted that, in those cells exposed to NRG1\( \beta \), the peptide was removed by washing the cells before the addition of the (-)-isoproterenol at the commencement of the 3-h secretory period. Thus the level of secretion in the absence

![Graph](image)

**Fig. 5.** Effects of NRG1\( \beta \) and leptin on the secretion of surfactant phospholipids from cultured fetal type II pneumocytes. After 3 days in culture, fetal rat type II pneumocytes were exposed to 24 h to 1.0 \( \mu \)Ci/ml [methan-\( ^3 \)H]choline chloride to facilitate labeling of the cellular phospholipids. The level of surfactant phospholipid secretion was ascertained after exposure of the cells to either 50 ng/ml NRG1\( \beta \) (gray bars); 20, 50, and 100 ng/ml leptin (hatched bars); or an equivalent volume of vehicle (control, white bars). The results are depicted as means \( \pm \) SE of 5 separate experiments, and any significant differences are denoted with an asterisk \(( *P < 0.05 \)).

| Table 1. Expression of the \( \beta \)-adrenergic receptor gene in lung type II cells after exposure to NRG1\( \beta \) |
|-----------------|-----------------|-----------------|-----------------|
| Exposure Time, h | 20              | 50              | 100             |
| NG1\( \beta \)   |                 |                 |                 |
| 2               | 1.28 ± 0.03 (4) | 0.80 ± 0.16 (6) | 0.97 ± 0.43 (4) |
| 4               | 1.06 ± 0.13 (4) | 0.99 ± 0.08 (6) | 0.94 ± 0.24 (4) |
| 6               | 1.18 ± 0.27 (5) | 1.26 ± 0.27 (5) | 1.25 ± 0.30 (5) |

The level of \( \beta \)-AR mRNA was determined using multiplex qPCR, and the data represent means \( \pm \) SE of the indicated no. of independent type II cells cultures in parentheses. Cultured fetal rat type II cells were exposed to serum-free minimal essential medium containing 20, 50, or 100 ng/ml of neuregulin-1\( \beta \) (NRG1\( \beta \)) or an equivalent volume of water (control). After the indicated exposure time, the cells were subjected to RNA extraction, and the mRNA concentration of these extracts was quantified.

![Graph](image)

**Fig. 6.** Effect of NRG1\( \beta \) on \( \beta \)-AR gene expression in type II pneumocytes. Type II cells were exposed to 20, 50, and 100 ng/ml of NRG1\( \beta \) or an equivalent volume of vehicle (control) with (black bars) or without (gray bars) 50 nM dexamethasone. After 8 h the cells were harvested, and the mRNA extracted followed by its quantification using an Agilent 2100 Bioanalyzer. Multiplex qPCR was carried out using both GAPDH and \( \beta \)-AR primers and probes. The level of \( \beta \)-AR gene expression relative to that in control cultures was ascertained using the method of Pfaffl (29). The results represent means \( \pm \) SE of 5 separate experiments, and significant differences from cells incubated with dexamethasone alone are indicated with asterisks \(( *P < 0.05 \) and \( ***P < 0.001 \)).
of (−)-isoproterenol was the same in cells exposed to either NRG1β or vehicle (control).

As was the case with induction of β-AR, NRG1β-induced elevation in the secretory response to (−)-isoproterenol was the same in type II cells derived from either male or female fetuses (Fig. 4B). Such a finding is in contrast to that observed in cells exposed to media that had been conditioned by lung fibroblasts, where the response to (−)-isoproterenol was greater when the type II cells were derived from female fetuses (Fig. 1B). This suggests that this sex-linked difference can be attributed to the fibroblasts, which is consistent with the previously published conclusions of Torday (38) and Floros et al. (15).

Previous studies have shown that both leptin (20, 39) and NRG1β (10) stimulate the rate of synthesis of surfactant components, including disaturated phosphatidylcholine, in fetal type II pneumocytes. This prompted us to investigate the effect of these two peptides on surfactant phospholipid secretion. Although leptin, at concentrations of up to 100 ng/ml, significantly elevates surfactant phospholipid synthesis (39), it has no effect on the level of its secretion from type II cells (Fig. 5). In contrast, NRG1β significantly elevated not only the synthesis (3.1-fold; P < 0.05) but also the secretion of surfactant phospholipids (2.5-fold; P < 0.05, see Figs. 3B and 5) from these cells. Thus, NRG1β has a multifaceted effect on type II pneumocytes, which leads to a substantial increase in the production of surfactant components.

Effects of dexamethasone and NRG1β on β-AR gene expression in type II pneumocytes. Multiplex qPCR was carried out using both GAPDH and β-AR RT-PCR primers together with specific fluorescently tagged TaqMan probes for each of the RT-PCR products. When type II cells were exposed to 20, 50, and 100 ng/ml NRG1β for 2, 4, or 6 h and the relative level of β-AR gene expression determined, using the Pfaffl method (29), no significant effect of NRG1β was observed (Table 1). When type II cells were likewise exposed to 50 nM dexamethasone for 8 h there was a twofold increase in the level of expression of the β-AR gene. Moreover, although there was no significant effect of NRG1β alone, the β-AR mRNA level in cells exposed to either 50 or 100 ng/ml NRG1β in combination with dexamethasone was significantly greater than in cells treated with steroid alone (P < 0.05 and P < 0.001, respectively) (Fig. 6). It is therefore apparent that NRG1β and dexamethasone act synergistically in their ability to enhance the level of β-AR mRNA within type II pneumocytes. The data also suggest that the two agents operate via different but complementary mechanisms. One possibility is that NRG1β may stabilize the β-AR mRNA, but only when expression was enhanced by exposure to glucocorticoids.

An anomalous result was the finding that exposure of type II cells to NRG1β for 24 h enhanced the β-AR activity (Fig. 4A) despite this treatment having no significant effect on the level of β-AR gene expression at 2, 4, 6, or 8 h (Table 1 and Fig. 6). There are at least two possibilities that could account for this apparently contradictory observation. The upregulation of β-AR activity by NRG1β may be due to a transient elevation in the expression of the β-AR gene at some time after 8 h exposure to the peptide, leading to an enhanced activity of β-AR at 24 h. This suggestion is consistent with the observations that dexamethasone induces a transient elevation in the β-AR mRNA at 2 h but an enhanced β-AR activity 22 h later in adult lung tissue from both humans (24) and rats (26). Alternatively, because the β-AR assay is measuring surface labeling of β-AR, the increase in surface β-AR activity upon stimulation of cells with NRG1β could be due to enhanced recruitment of β-AR receptors to the cell surface from internal vesicles. Such a mechanism would not require an increase in mRNA expression to elevate β-AR surface labeling.

In conclusion, it is evident from this study that the extent of glucocorticoid enhancement of β-AR activity and of (−)-isoproterenol-induced secretion of surfactant phospholipids in type II cells is greater in the presence of FCM. Given that FCM has been shown to contain NRG1β, it is relevant that this peptide also stimulates both β-AR activity and (−)-isoproterenol-induced secretion of surfactant phospholipids. Unlike the effects generated by glucocorticoids, NRG1β induces virtually identical responses in type II cells derived from male or female fetuses. The observation that the response to glucocorticoids is greater in the presence of NRG1β is consistent with the finding that the glucocorticoid-induced stimulation of β-AR gene expression is also enhanced by NRG1β. Because the effects of NRG1β on lung maturation are wide ranging, causing increased surfactant synthesis and secretion and stimulating the activity of β-AR, it is suggested that the administration of NRG1β might provide an improved treatment regime for neonatal respiratory distress syndrome.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: G.K., J.E.D., M.H.C., D.B., and G.L.M. conception and design of research; G.K. and J.E.D. performed experiments; G.K., J.E.D., M.H.C., and D.B. analyzed data; G.K., J.E.D., M.H.C., and G.L.M. interpreted results of experiments; G.K., J.E.D., and M.H.C. prepared figures; G.K., M.H.C., and G.L.M. drafted manuscript; J.E.D., M.H.C., D.B., and G.L.M. edited and revised manuscript; M.H.C. and G.L.M. approved final version of manuscript.

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