Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4357–4361, May 1995
Developmental Biology

Bombesin and [Leu⁸]phyllolitorin promote fetal mouse lung branching morphogenesis via a receptor-mediated mechanism

(embryogenesis/gastrin-releasing peptide/reverse PCR/*in situ* hybridization/fibronectin)

KATHERINE A. KING*, JOHN S. TORDAY†, AND MARY E. SUNDAY‡§

*Department of Pediatrics, Children's Hospital and Harvard Medical School, Boston, MA 02115; †Department of Pediatrics, University of Maryland Medical School, Baltimore, MD 21201; and ‡Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Communicated by Judah Folkman, Harvard Medical School, Boston, MA, January 3, 1995 (received for review May 21, 1993)

ABSTRACT **Pulmonary neuroendocrine cells are localized predominantly at airway branchpoints. Previous work showed that gastrin-releasing peptide (GRP), a major pulmonary bombesin-like peptide, occurred in neuroendocrine cells exclusively in branching human fetal airways. We now demonstrate that GRP and GRP receptor genes are expressed in fetal mouse lung as early as embryonic day 12 (E12), when lung buds are beginning to branch. By *in situ* hybridization, GRP receptor transcripts were at highest levels in mesenchymal cells at cleft regions of branching airways and blood vessels. To explore the possibility that bombesin-like peptides might play a role in branching morphogenesis, E12 lung buds were cultured for 48 hr in serum-free medium. In the presence of 0.10–10 μM bombesin, branching was significantly augmented as compared with control cultures, with a peak of 94% above control values at 1 μM ($P < 0.005$). The bombesin receptor antagonist [Leu¹³-ψ(CH₂NH)Leu¹⁴]bombesin alone (100 nM) had no effect on baseline branching but completely abolished bombesin-induced branching. A bombesin-related peptide, [Leu⁸]phyllolitorin also increased branching (65% above control values at 10 nM, $P < 0.005$). [Leu⁸]Phyllolitorin also significantly augmented thymidine incorporation in cultured lung buds. Fibronectin, which is abundant at branchpoints, induces GRP gene expression in undifferentiated cell lines. These observations suggest that BLPs secreted by pulmonary neuroendocrine cells may contribute to lung branching morphogenesis. Furthermore, components of branchpoints may induce pulmonary neuroendocrine cell differentiation as part of a positive feedback loop, which could account in part for the high prevalence of these cells at branchpoints.**

Pulmonary neuroendocrine (NE) cells containing bombesin-like peptide (BLP) immunoreactivity are present at high numbers in human newborn and fetal lung (1, 2). BLPs include amphibian bombesin and mammalian/amphibian gastrin-releasing peptide (GRP) (3, 4), in which the bioactive amidated carboxyl-terminal amino acid sequence is -His-Leu-Met-NH_2. BLPs have been demonstrated by several laboratories to be potent mitogens for many small-cell lung carcinomas (SCLCs) (5, 6), normal adult bronchial epithelial cells (7), and several fibroblast cell lines, including adult human pulmonary fibroblasts (8, 9). Early descriptive studies of human fetal lung demonstrated that GRP gene expression proceeded in a proximal-to-distal pattern in parallel with the growth of the airways (10). Cumulatively, these early observations suggested that BLPs might play a role in normal fetal lung development (3).

To explore the role of BLPs in lung development, bombesin was administered to cultured human and murine fetal lung explants and fetal mice *in utero*. The results demonstrated increased thymidine incorporation into nuclear DNA, increased choline incorporation into surfactant phospholipids, and increased relative numbers of type II pneumocytes by electron microscopy (11). A blocking monoclonal antibody to BLP (2A11) (5) inhibited automaturation of fetal lung (11, 12). Potentiation of levels of endogenous BLPs by inhibiting their degradation led to increased fetal lung growth and maturation (13, 14), including elevated surfactant apoprotein transcript levels and increased surfactant secretion in mice *in utero* (14).

The phyllolitorins, a second amphibian peptide family related to BLPs, have the amidated carboxyl-terminal sequence -Ser-(Leu/Phe)-Met-NH_2 (15, 16). Amino acid analysis of BLPs present in bronchoalveolar lavage fluid from smokers suggested the presence of a mammalian phyllolitorin-like peptide (17). Mammalian phyllolitorins might be expected to have functions similar to BLPs because these peptides are immunologically crossreactive (ref. 17 and M.E.S., unpublished data). Both bombesin and phyllolitorins can act as bronchoconstrictors (18, 19). To date, no further information has been reported regarding the functional implications of a mammalian phyllolitorin.

In his first description of pulmonary NE cells, Feyrter (20) noted a high prevalence of these cells at airway branchpoints. Detailed morphometric studies by Cutz and coworkers (21) localized 50–60% of serotonin-positive pulmonary NE cells at branchpoints in three-dimensional reconstructions of fetal rabbit lung. Analyses of GRP gene expression in human fetal pulmonary NE cells showed that cells containing GRP transcripts occurred exclusively in airways that had begun to branch, in particular in early (8–9 weeks of gestation) lung development (10).

The aim of the present study was to explore potential involvement of BLPs in embryonic lung branching morphogenesis and growth, with our initial focus on GRP, a major pulmonary BLP. We first assessed the temporal and spatial distribution of GRP gene expression in developing murine embryonic lung. Functional effects of BLPs on branching morphogenesis of cultured lung buds were then evaluated. The importance of mesenchyme for morphogenesis is well recognized, with fibronectin and other extracellular matrix components localized predominantly at airway branchpoints (22). Fibronectin and laminin have been implicated in lung branching morphogenesis because specific blocking peptides or monoclonal antibodies inhibit branching (23, 24). For these reasons, we have also begun to explore the ability of fibronectin to stimulate GRP gene expression by undifferentiated SCLC cell lines.

MATERIALS AND METHODS

Animals and RNA Preparations. Timed pregnant outbred CD1 mice were obtained from Charles River Breeding Laboratories at gestational day 9 (embryonic day 9, E9). Lungs were dissected from E12 to E18 fetal mice and from neonatal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BLP, bombesin-like peptide; E*n*, embryonic day *n*; GRP, gastrin-releasing peptide; L13BN, [Leu¹³-ψ(CH₂NH)Leu¹⁴]-bombesin; L8PL, [Leu⁸]phyllolitorin; NE, neuroendocrine; RT, reverse transcription; SCLC, small-cell lung carcinoma.

§To whom reprint requests should be addressed.

mice. Total RNA was prepared from freshly harvested fetal lung tissues by the guanidinium isothiocyanate method (25).

***In Situ* Hybridization.** *In situ* hybridization was performed (26) with 5 μm sections and 20 μl of ^{35}S-labeled antisense cRNA probe or sense cRNA probe at 5×10^4 cpm/μl in hybridization buffer. Radiolabeled probes were prepared as described (27).

Polymerase Chain Reaction (PCR) on Reverse-Transcribed RNA. First-strand cDNA was prepared by reverse transcription (RT) of 10 μg of total RNA Moloney with murine leukemia virus reverse transcriptase (2 units; Bethesda Research Laboratories) in the presence of human placental RNase inhibitor (5 units; Sigma) and random hexamer primers [5′-p(dN)$_6$, 1 unit; Pharmacia LKB]. Oligodeoxynucleotides for PCR were made with an Applied Biosystems 381A DNA synthesizer. PCR primers were designed to span at least one intron to ensure that any contaminating genomic DNA would yield a higher molecular weight band: rat GRP [precursor peptide aa 20–116, insert 306 bp; 5′-GGCAGCCACTGGGCTGTA-GGACACTTAATG-3′; 3′-GAGAACCTGGAGCAGAGA-GTCTACCAACTT-5′ (28)]; mouse GRP receptor [precursor peptide aa 1–233, insert 700 bp; 5′-ATGGCTCCAAATAAT-TGTTCC-3′; 3′-ATGAAGTAGTAGTAGACAGAG-5′ (29)]; and mouse β-actin [aa 27–126, insert 300 bp; 5′-GTGGGCCGCTCTAGGCACCA-3′; 3′-TGGCCTTAGG-GTTCAGGGGG-5′ (30)]. The sequences of internal primers which were used for probing PCR Southern blot were as follows: for rat GRP, 5′-TACATCCGCTGGGAAGAAG-CTGCAAGGAAT-3′; for mouse GRP receptor, 5′-CCCCA-CTGAAGTAAGTTGTAT-3′; for β-actin, 5′-AACTGG-GACGACATGGAGAAGATCTGGCAC-3′.

PCR used 1 μl of cDNA in a standard reaction mixture (31) and consisted of 40 cycles for GRP (nonquantitative), 35 cycles for GRP (semiquantitative), 35 cycles for the GRP receptor (semiquantitative), or 25 cycles for actin (semiquantitative), with each cycle consisting of denaturation (0.5 min, 93°C), annealing (1.0 min, 50°C), and extension (1.5 min, 72°C). The PCR products were analyzed by Southern blots prepared by standard techniques and hybridized with probes labeled by phage T4 kinase (25) using 30-bp primers corresponding to internal cDNA sequence of each gene. Using 35 cycles for GRP and GRP receptor transcripts and 25 cycles for actin, we were within the linear range of detection. Increased positive control RNA (0.05, 0.5, and 5.0 μg in a 20-μl RT reaction) samples were used to actually demonstrate a linear increase in RT–PCR signals. Therefore, we observed a linear correlation between the relative amounts of given mRNAs present in the products of the same RT reaction, which were then normalized for the relative amounts of actin transcripts as a control reference "housekeeping" mRNA (31).

E12–14 Murine Lung Bud Cultures. Embryos from day 12 timed pregnant mice were removed aseptically into Petri dishes containing α-minimal essential medium (α-MEM) (Mediatech) with 10% fetal bovine serum (GIBCO/BRL). Lung buds isolated under a dissecting microscope were washed in serum-free medium and transferred to a semipermeable membrane (Falcon cell inserts with 0.8-μm pore size). Cell inserts were positioned overlying 0.5 ml of serum-free BGJb medium (Fitton-Jackson modification; GIBCO/BRL). Bombesin, [Leu^8]phyllolitorin (L8PL), and the specific bombesin receptor antagonist [Leu^{13}-ψ(CH$_2$NH)Leu^{14}]bombesin (L13BN) (Peninsula Laboratories) were added in the appropriate concentrations to individual wells (3, 16, 32). Lung buds were photographed *in situ* before addition of reagents to the medium on day 0 (E12) and again after 48 hr of culture ("E14"). Branchpoints were counted along the periphery of the cultured buds by two observers (K.A.K. and M.E.S.) without knowledge of the experimental groups. Results were expressed as percent increase in branching in 48 hr above baseline (medium alone), which was defined as zero. For thymidine incorporation assays, lung buds were submerged in 1.0 ml of serum-free α-MEM for 48 hr, with [*methyl*-^{3}H]thymidine (5 μCi/ml; 1 μCi = 37 kBq) added for the final 4 hr, after which time individual buds were harvested for analyses of thymidine incorporation as described (11). DNA content was assayed in pools of 12 buds (necessary for being within the linear range of detection) as described (11).

Tissue Culture. Tissue culture flasks were coated overnight with bovine plasma fibronectin (GIBCO/BRL). The undifferentiated SCLC cell line H82 (33) was cultured for 24 hr in RPMI 1640 medium containing 10% fetal bovine serum (GIBCO/BRL) on uncoated and fibronectin-coated plates. Cells were harvested by centrifugation and RNA was prepared for RT–PCR as described above.

RESULTS

RNA Analyses by RT–PCR. Previous studies using Northern blot analyses with poly(A)$^+$ RNA from fetal mouse lung showed that GRP transcripts were detectable from E16 to E18, peaking on E18 (11). To determine whether the GRP gene (28) is expressed in early lung development, RT–PCR was carried out using 40 cycles of PCR, which is above the linear range of detection (30–35 cycles) for GRP (31). GRP transcripts were detectable (Fig. 1) from E12 to E18 and in neonatal lung [postnatal day 1 (P1) to P14]; GRP transcripts were undetectable in adult lung, fetal liver, and H82 variant SCLC under these conditions (ref. 31 and unpublished data). Expression of the GRP receptor gene (29) in developing fetal lung was assessed by semiquantitative RT–PCR conditions (Fig. 1). GRP receptor transcripts were present from E12 to E18 and for 1 day after birth, peaking at E13 and E15–17, but thereafter dropped off to undetectable levels. β-Actin transcripts were at comparable levels in all reverse-transcribed samples (Fig. 1).

***In Situ* Hybridization of GRP Receptor Transcripts.** *In situ* hybridization using GRP receptor antisense cRNA probes (27, 29) demonstrated high levels of GRP receptor transcripts in mesenchymal tissues surrounding developing airways and blood vessels in mouse fetal lung from E14 to E18. Representative sections are shown in Fig. 2 *A* (brightfield) and *B* (darkfield). The most intense hybridization was present at the branchpoint clefts of both airways and vasculature (Fig. 2*B*, arrows). The hybridization signal was stronger in mesenchyme associated with the vasculature, as compared with the airways (unpublished data). The same lungs probed in parallel with the GRP receptor sense probe did not give a hybridization signal (Fig. 2 *C* and *D*).

E12–14 Murine Lung Bud Cultures. Lung buds were dissected at E12, when >90% of these buds had four to nine peripheral branchpoints (Fig. 3*A*). After culture for 48 hr in serum-free medium, the number of peripheral branches generally increased by a factor of 1.5–3 (Fig. 3*B*).

E12 lung buds were treated for 48 hr with various doses of bombesin. The results of 15 experiments were pooled and are summarized in Fig. 4. Bombesin treatment of lung buds (3–5 buds per group per experiment) induced a significant increase in branching morphogenesis at 100 nM [36 ± 8% (65 buds), $P < 0.001$], 1 μM [93 ± 27% (7 buds), $P < 0.001$], and 10 μM [55 ± 22% (8 buds), $P < 0.01$] when compared with control

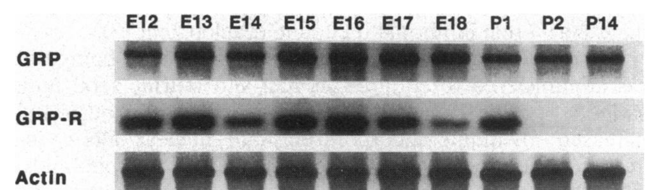

FIG. 1. GRP and GRP receptor transcripts in fetal mouse lung. Lungs were harvested from untreated fetal mice at E12-E18 and from neonatal mice on postnatal day 1 (P1), P2, and P14. Southern blots of the PCR products were probed for GRP, GRP receptor, and β-actin transcripts.

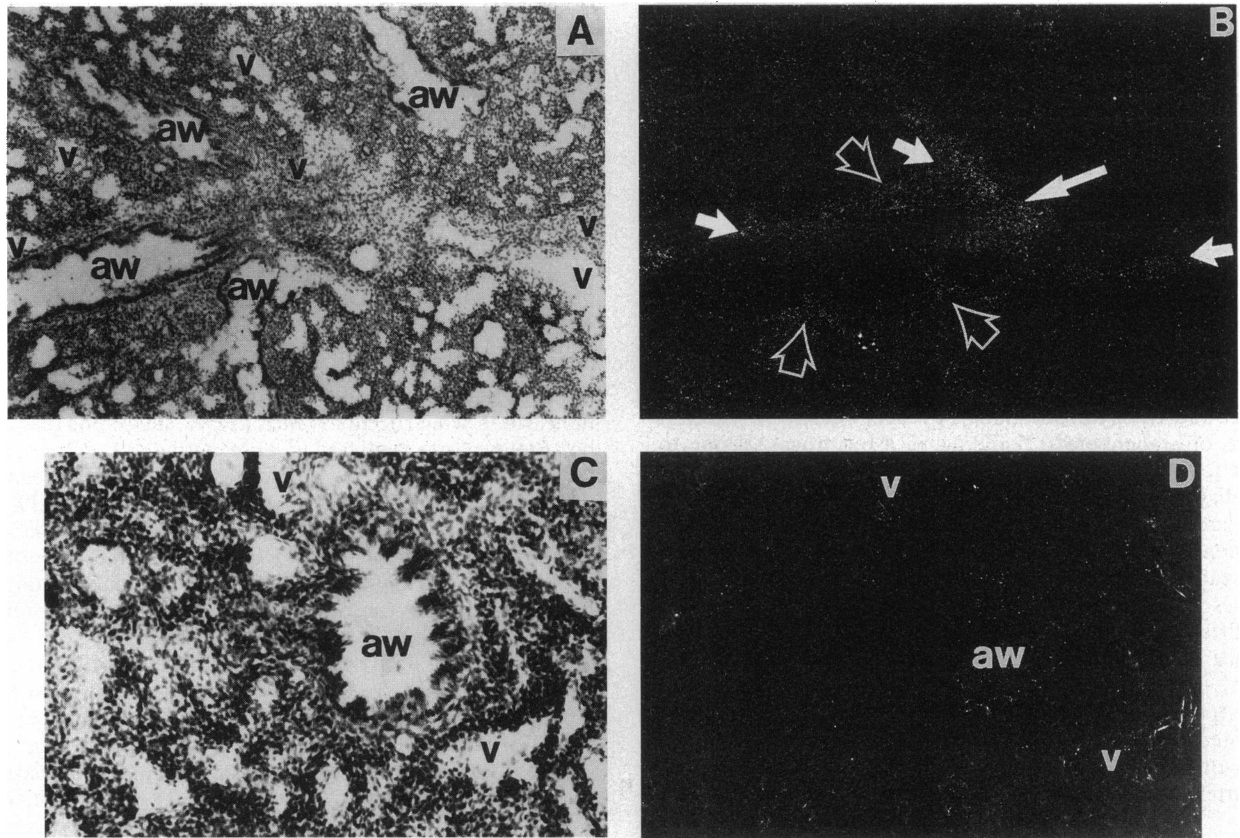

FIG. 2. *In situ* hybridization of fetal mouse lung for GRP receptor transcripts. Each pair of photographs represents the same field viewed by brightfield microscopy to demonstrate histology (*A* and *C*) and darkfield microscopy to demonstrate autoradiographic grains (*B* and *D*). (*A* and *B*) Probed with GRP receptor antisense cRNA. Hybridization is most intense in mesenchyme of the cleft regions of airways (open arrows), blood vessels (small solid arrows), and a region with branching airways and blood vessels (long solid arrow). aw, Airway; v, blood vessel. (×26.) (*C* and *D*) Probed with control GRP receptor sense cRNA. (×70.)

buds cultured with medium alone, the mean of which was defined as baseline for each experiment [0 ± 7% (68 buds total)]. The bombesin receptor antagonist L13BN (100 nM) administered with bombesin (100 nM) completely blocked the bombesin-induced increase in branching morphogenesis [0 ± 7% (17 buds), $P < 0.05$ compared with 100 nM bombesin alone]. When L13BN was administered alone, no effect was elicited. These observations are consistent with previous studies of growth and maturation in E18 fetal mouse lung (13, 14).

In immunoperoxidase analyses of human fetal lung, we were able to absorb all of the immunostaining of pulmonary NE cells by anti-bombesin antiserum (17) by using either bombesin or the bombesin-related crossreactive peptide L8PL at 1 μg/ml (unpublished data). To determine whether L8PL could elicit a physiological response similar to bombesin in early lung development, E12 lung buds were treated for 48 hr with various doses of L8PL. The pooled results of nine experiments are given in Fig. 5. Peak branching occurred at 10 nM L8PL [65 ± 17% (29 buds), $P < 0.001$], with a significant effect also observed at 100 nM [42 ± 20% (36 buds), $P < 0.016$].

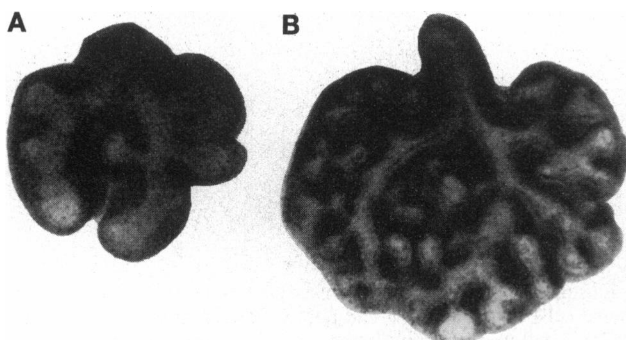

FIG. 3. Embryonic mouse lung bud cultures. Lung buds were dissected on E12 and cultured for 48 hr. (*A*) E12 lung bud has eight peripheral branchpoints. (×26.) (*B*) After 48 hours of culture, the same lung bud (E14) has 23 peripheral branchpoints. (×26.)

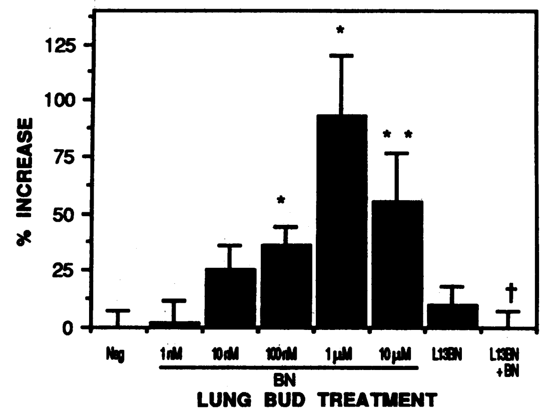

FIG. 4. Quantitation of lung branching morphogenesis in serum-free medium with varying doses of bombesin (BN) or the specific bombesin receptor antagonist L13BN. E12 lung buds were cultured in medium alone (Neg, negative) or in medium containing 1 nM to 10 μM bombesin (BN), 100 nM L13BN, or 100 nM L13BN plus 100 nM BN. Results of 15 experiments were pooled and are expressed as percent increase in branching above baseline. *, $P < 0.001$; **, $P < 0.01$ compared with the negative control (Neg, medium alone); †, $P < 0.05$ compared with 100 nM bombesin alone.

In addition, L8PL stimulated thymidine incorporation in E12–14 lung buds, with a peak effect at 100 nM (Table 1). The data were normalized so that the negative control was defined as baseline, equal to zero; in each group of each experiment there were three complete lung buds, so that each data point represents a pool of one bud from each of three litters. In contrast, 100 nM bombesin had no effect on thymidine incorporation. The effect of L8PL was similar when lung buds from three other experiments were pooled to generate sufficient tissue to determine DNA content (4 buds from each of three litters per group, for a total of 12 buds per group): untreated control, 2301 dpm/nmol of DNA; 100 nM L8PL: 2919 dpm/nmol of DNA (27% increase over baseline); 5% fetal bovine serum, 3366 dpm/nmol of DNA (46% increase over baseline).

BLPs are known to stimulate pulmonary fibroblast growth (9). We addressed the question of whether fibronectin (in cleft regions of branchpoints) could act in a reciprocal fashion to induce features of NE differentiation in undifferentiated lung epithelial cells. We used an undifferentiated SCLC line, H82 (33), that is capable of undergoing terminal NE differentiation in response to retinoic acid (34). H82 was cultured for 24 hr in tissue culture flasks precoated with fibronectin, and RT-PCR analyses were carried out for GRP gene induction. The well-differentiated SCLC cell line H345, which is known to produce high levels of BLPs (33), was used as the positive control. Preliminary analyses showed high levels of GRP transcripts in H345 cells but no detectable GRP transcripts in untreated H82 cells (Fig. 6, lanes 1 and 2). After fibronectin treatment, high levels of GRP gene expression were induced in H82 after 24 hr (Fig. 6, lane 3).

DISCUSSION

This study demonstrates that bombesin and the bombesin-related peptide L8PL augment branching morphogenesis in E12 mouse lung buds in serum-free medium in a dose-dependent fashion. These data suggest that pulmonary NE cells may play an active role in promoting branching morphogenesis during fetal lung development, via the synthesis and secretion of bioactive BLPs such as GRP acting at least in part via GRP receptors. Others have noted a similar trend of stimulation of branching by bombesin in serum-containing medium (35). The timing of the branching effect coincides with

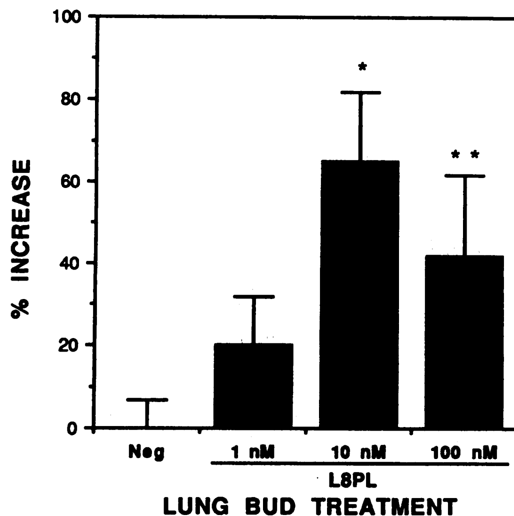

FIG. 5. Quantitation of lung branching morphogenesis in serum-free medium with various doses of L8PL. E12 lung buds were cultured in medium alone (Neg) or in medium containing 1–100 nM L8PL. Results of nine experiments were pooled and are expressed as percent increase in branching above baseline. *, $P < 0.001$; **, $P < 0.01$ compared with the negative control (Neg, medium alone).

Table 1. [^{3}H]Thymidine incorporation in 48-hr lung bud cultures

Treatment	% increase over negative control	P value
Negative control	0 ± 0 (3)	—
Bombesin (100 nM)	10.5 ± 17.5 (3)	0.581
L8PL (10 nM)	28.3 ± 8.4 (3)*	0.028
L8PL (100 nM)	60.0 ± 8.6 (3)**	0.002

Lung buds were harvested from timed pregnant mice on E12. The data shown represent pooled results from three experiments, normalized so that the untreated group is defined as baseline (zero). In each experiment, [^{3}H]thymidine incorporation was determined for a pool of three lung buds, one bud from each of the same three litters. Values are given as mean ± SEM, with the number of experiments given in parentheses. *, $P < 0.028$; **, $P < 0.002$.

the expression of the GRP and GRP receptor genes in fetal mouse lung, which occurs as early as E12, suggesting that it may be at least partly mediated by GRP acting at GRP receptors. Of three cloned BLP receptor genes, only the GRP receptor is known to be expressed in normal lung (29, 36–39). An additional candidate is a BLP-binding protein which may represent another receptor, cloned from E18 fetal mouse lung (40). The predominant receptor in embryonic mouse lung may be either the GRP receptor or an as yet undescribed subtype, such as a phyllolitorin receptor.

The demonstration that the highest levels of GRP receptor transcripts occur in mesenchymal cells near large airways and blood vessels is in keeping with observations of others in a model of rat lung development (27). These data suggest that the mesenchyme may be a critical component in the cascade of events leading to increased branching. The predominant localization of GRP receptor transcripts at cleft regions of branchpoints suggests that local gradients of receptor gene expression may be implicated in the branching process, in addition to the predominant localization of pulmonary NE cells as a point source of BLPs in this same region.

We observed stimulation of branching morphogenesis with peak effects at 1 μM bombesin and/or 10 nM L8PL. Thymidine incorporation was also stimulated by 10–100 nM L8PL, but not by 100 nM bombesin. In contrast, BLPs stimulate fetal lung cell proliferation and type II cell differentiation on E18, with peak effects observed at 1 nM bombesin in lung organ cultures (11, 12, 14). These data suggest that the events in early embryonic mouse lung development may be partially mediated by different BLP ligands and/or receptors. The induction of branching by BLPs in our system is completely blocked by the specific bombesin receptor antagonist L13BN, but this antagonist could block a novel

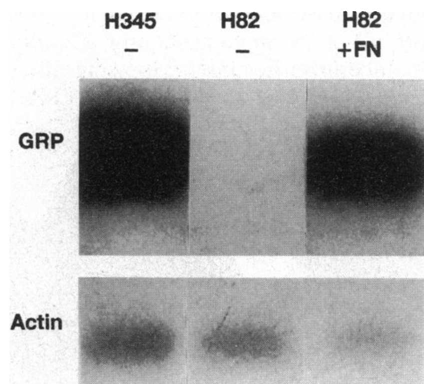

FIG. 6. Induction of GRP transcripts in cultured pulmonary epithelial cells by fibronectin. The poorly differentiated SCLC cell line H82 was cultured for 24 hr in uncoated tissue culture flasks (−) or in flasks that had been precoated with bovine plasma fibronectin (+FN). Note the absence of GRP transcripts in untreated H82 and strong induction of GRP gene expression in fibronectin-treated cells. The classic SCLC cell line H345 was used as a positive control, because this cell line is known to produce high levels of GRP (33).

phyllolitorin receptor as well. Note that 100 times as much bombesin is required to achieve the same effect on branching as L8PL, suggesting that induction of branching may be primarily a phyllolitorin-mediated effect. The observation of highly significant branching at low concentrations of L8PL suggests that a yet-to-be-identified mammalian phyllolitorin and/or phyllolitorin receptor may be implicated in these processes. Amino acid analyses of BLPs in bronchoalveolar lavage fluid from human smokers have already suggested the presence of a mammalian pulmonary phyllolitorin (17).

Mechanisms for BLP-induced branching are likely to include regional cell proliferation, altered cell adhesion, or cell motility. Studies of fetal rabbit lung development demonstrated that GRP induced increased [^{3}H]thymidine labeling of non-NE pulmonary cells but not of pulmonary NE cells (41). BLPs may promote branching also in part by stimulating production of extracellular matrix components such as fibronectin or laminin (23, 24). Our demonstration of induction of GRP gene expression in undifferentiated SCLC cells by exposure to fibronectin-coated plates suggests that fibronectin may induce the NE phenotype in undifferentiated precursors, creating a positive paracrine feedback loop with cell differentiation taking place in parallel with branching morphogenesis. Extracellular matrix components, including fibronectin and laminin, have been implicated in the differentiation of other cell types, including NE cells (39, 42). Fibronectin and laminin have been implicated in lung branching morphogenesis because specific blocking peptides or monoclonal antibodies inhibit branching (23, 24). Thus, pulmonary NE cell differentiation and the formation of branchpoints may be interdependent, with reciprocal induction occurring during development. In addition, BLPs may directly alter epithelial cell migration. Bombesin has been reported to stimulate chemotaxis of macrophages and SCLC (epithelial) cell lines (43). BLPs may trigger the release of a soluble branching factor such as hepatocyte growth factor (44) or basic fibroblast growth factor (45) from pulmonary fibroblasts or primitive mesenchymal cells, which could act in a secondary manner to promote branching by altering cell migration. In conclusion, the present study suggests that the localization of the majority of pulmonary NE cells at airway branchpoints may be related to the secretion of peptides such as BLPs. A chemical gradient of BLPs and peptide receptors, in turn, may stimulate cell proliferation and local mesenchymal processes such as fibronectin production, leading to branching morphogenesis of airways and blood vessels.

We thank Kevin Hooper and Dr. Merton Bernfield for helpful discussions and assistance in teaching our laboratory the methodology for lung bud cultures and Dr. Richard Maas for the generous use of his dissecting photomicroscope. This work was supported by National Institutes of Health grant RO1-HL44984 (M.E.S.), the Children's Hospital Pediatric Pulmonary Training Grant (K.A.K.), and a grant-in-aid from the American Heart Association (J.S.T.).

1. Wharton, J., Polak, J. M., Bloom, S. R., Ghatei, M. A., Solcia, E., Brown, M. R. & Pearse, A. G. E. (1978) *Nature (London)* **273**, 769–770.
2. Lauweryns, J. M. & Peuskens, J. C. (1969) *Life Sci.* **8**, 577–585.
3. Sunday, M. E., Kaplan, L. M., Motoyama, E., Chin, W. W. & Spindel, E. R. (1988) *Lab. Invest.* **59**, 5–24.
4. Nagalla, S. R., Gibson, B. W., Tanf, D., Reeve, J. R., Jr. & Spindel, E. R. (1992) *J. Biol. Chem.* **267**, 6916–6922.
5. Cuttitta, F., Carney, D. N., Mulshine, J., Moody, T. W., Fedorko, J., Fischler, A. & Minna, J. D. (1985) *Nature (London)* **316**, 823–826.
6. Weber, S., Zuckerman, J. E., Bostwick, D. G., Bensch, K. G., Sikic, B. I. & Raffin, T. A. (1985) *J. Clin. Invest.* **75**, 306–309.
7. Siegfried, J. M., Guentert, P. J. & Gaither, A. L. (1993) *Anat. Rec.* **236**, 241–247.
8. Rozengurt, E. & Sinnett-Smith, J. (1993) *Proc. Natl. Acad. Sci. USA* **80**, 2936–2940.
9. Aguayo, S. M., King, T. E., Waldron, J. A., Sherritt, K. M., Kane, M. A. & Miller, Y. E. (1990) *J. Clin. Invest.* **86**, 838–844.
10. Spindel, E. R., Sunday, M. E., Hofler, H., Wolfe, H. J., Habener, J. F. & Chin, W. W. (1987) *J. Clin. Invest.* **80**, 1172–1179.
11. Sunday, M. E., Hua, J., Dai, H. B., Nusrat, A. & Torday, J. S. (1990) *Am. J. Respir. Cell Mol. Biol.* **3**, 199–205.
12. Sunday, M. E., Hua, J., Reyes, B., Masui, H. & Torday, J. S. (1993) *Anat. Rec.* **236**, 25–32.
13. Sunday, M. E., Hua, J., Torday, J., Reyes, B. & Shipp, M. A. (1992) *J. Clin. Invest.* **90**, 2517–2525.
14. King, K. A., Hua, J., Torday, J. S., Drazen, J. M., Graham, S. A., Shipp, M. A. & Sunday, M. E. (1993) *J. Clin. Invest.* **91**, 1969–1973.
15. Lazarus, L. H., Wilson, W. E., Gaudino, G., Irons, B. J. & Guglietta, A. (1985) *Peptides* **6**, 295–307.
16. Yasuhara, T., Nakajima, T., Nokihara, K., Yanaihara, C., Yanaihara, N., Erspamer, V. & Erspamer, G. F. (1983) *Biomed. Res.* **4**, 407–412.
17. Aguayo, S. M., Kane, M. A., King, T. E., Schwarz, M. I., Grauer, L. & Miller, Y. E. (1989) *J. Clin. Invest.* **84**, 1105–1113.
18. Impicciatore, M. & Bertaccini, G. (1973) *J. Pharm. Pharmacol.* **25**, 872–875.
19. Erspamer, G. F., Mazzanti, G., Farruggia, G., Nakajima, T. & Yanaihara, N. (1984) *Peptides* **5**, 765–768.
20. Feyrter, F. (1938) *Ueber diffuse endokrine epitheliale Organe* (Barth, Leipzig).
21. Cho, T., Chan, W. & Cutz, E. (1989) *Cell Tissue Res.* **255**, 353–262.
22. Infeld, M. D., Brennan, J. A. & Davis, P. B. (1993) *Am. J. Respir. Cell Mol. Biol.* **8**, 69–76.
23. Roman, J., Little, C. W. & McDonald, J. A. (1991) *Development (Cambridge, U.K.)* **112**, 551–558.
24. Schuger, L., O'Shea, S., Rheinheimer, J. & Varani, J. (1990) *Dev. Biol.* **137**, 26–32.
25. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York).
26. Sunday, M. E. (1991) *Methods Neurosci.* **5**, 123–136.
27. Wada, E., Battey, J. & Wray, S. (1993) *Mol. Cell. Neurosci.* **4**, 13–24.
28. Lebacq-Verheyden, A. M., Krystal, G., Sartor, O., Way, J. & Battey, J. F. (1988) *Mol. Endocrinol.* **2**, 556–563.
29. Battey, J. F., Way, J. M., Corjay, M. H., Shapira, H., Kusano, K., Harkins, R., Wu, J. M., Slattery, T., Mann, E. & Feldman, R. I. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 395–399.
30. Ng, S. Y., Gunning, P., Eddy, P., Ponte, P., Leavitt, J., Shows, T. & Kedes, L. (1985) *Mol. Cell. Biol.* **5**, 2720–2732.
31. Sunday, M. E. & Willett, C. G. (1992) *Cancer Res.* **52** Suppl., 2677S–2686S.
32. Coy, D. H., Heinz-Erian, P., Jiang, N.-Y., Sasaki, Y., Taylor, J., Moreau, J.-P., Wolfrey, W. T., Gardner, J. D. & Jensen, R. T. (1988) *J. Biol. Chem.* **263**, 5056–5060.
33. Carney, D. N., Gazdar, A. F., Bepler, G., Guccio, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H. & Minna, J. D. (1985) *Cancer Res.* **45**, 2913–2923.
34. Doyle, L. A., Giangiulo, D., Hussain, A., Park, H. J., Yen, R. W. C. & Borges, M. (1989) *Cancer Res.* **49**, 6745–6751.
35. Aguayo, S. M. & Roman, J. (1992) *Clin. Res.* **40**, 297 (abstr.).
36. Kane, M. A., Aguayo, S. M., Portanova, L. B., Ross, S. E., Holley, M., Kelley, K. & Miller, Y. E. (1991) *J. Biol. Chem.* **266**, 9486–9493.
37. Cardiff, R. D., Leder, A., Kuo, A., Pattengale, P. K. & Leder, P. (1993) *Am. J. Pathol.* **142**, 1199–1207.
38. Wada, E., Way, J., Shapira, H., Kusano, K., Lebacq-Verheyden, A. M., Coy, D., Jensen, R. & Battey, J. (1991) *Neuron* **6**, 421–430.
39. Fathi, Z., Corjay, M. H., Shapira, H., Wada, E., Benya, R., Jensen, R., Viallet, J., Sausville, E. A. & Battey, J. F. (1993) *J. Biol. Chem.* **268**, 5979–5984.
40. Geraci, M. W., Miller, Y. E., Escobedo-Morse, A. & Kane, M. A. (1992) *Clin. Res.* **40**, 304 A (abstr.).
41. Speirs, V., Bienkowski, E., Wong, V. & Cutz, E. (1993) *Anat. Rec.* **236**, 53–61.
42. DeSanti, A. M., McDowell, E. M. & Strum, J. M. (1992) *Tissue Cell* **24**, 853–868.
43. Ruff, M., Schiffmann, E., Terranova, V. & Pert, C. B. (1985) *Clin. Immunol. Immunopathol.* **37**, 387–396.
44. Montesano, R., Matsumoto, K., Nakamura, T. & Orci, L. (1991) *Cell* **67**, 901–908.
45. Klagsbrun, M. & D'Amore, P. A. (1991) *Annu. Rev. Physiol.* **53**, 217–239.
46. McGowan, S. E. (1992) *FASEB J.* **6**, 2895–2904.
47. Warburton, D., Seth, R., Shum, L., Horcher, P. G., Hall, F. L., Werb, Z. & Slavkin, H. C. (1992) *Dev. Biol.* **149**, 123–133.