Steroid Receptors in Human Lung Cancer

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ABSTRACT

We have determined that a significant incidence of specific high affinity receptors for androgen (AR), estrogen (ER), and glucocorticoid (GR) is present in normal adult human lung and bronchogenic carcinoma cytosols. In contrast, a limited number of tumor cytosols bound progesterone. Binding characteristics for each class of steroid hormones were similar to those reported for other steroid-responsive normal and neoplastic tissues. ER was evenly distributed between squamous cell and adenocarcinoma cytosols with a slightly lower affinity, but higher content than normal lung. In normal lung, GR resolved into two distinct binding components based on affinity using a dextran-coated charcoal assay. AR in squamous cell carcinomas behaved in a similar manner. This was not observed when hydroxyapatite was used to separate bound from free ligand. When AR affinity and content were stratified on the basis of tumor grade in squamous cell carcinoma, the most undifferentiated tumors had a lower AR content and higher affinity. In contrast, there was no differentiation of AR content or affinity based on tumor grade in adenocarcinoma where AR also did not resolve into two distinct groups based on binding affinity. Although related to tumor grade, AR incidence and content were not related to stage of disease. In adenocarcinoma, initial results suggest GR affinity and content were inversely related to degree of tumor differentiation, while GR content was slightly lower in poorly differentiated squamous cell carcinomas. GR content in squamous cell carcinoma increased slightly between Stages I and II and declined significantly in Stage II patients. This was not observed in adenocarcinoma, where GR content appeared to increase with stage of disease. Our results suggest that a significant incidence of specific, high affinity receptors for estrogen, androgen, and glucocorticoid is present in nonsmall cell carcinomas of the lung, which could provide a useful starting point for examining whether steroids influence the natural history of selected bronchogenic carcinomas.

INTRODUCTION

Steroids, particularly glucocorticoids and estrogens, regulate mammalian lung differentiation and maturation (1-4). Glucocorticoids accelerate the maturation of type II alveolar epithelial cells which produce surfactant (5) and induce protein synthesis (6, 7) in rabbit lung. The effects of glucocorticoids in rat and rabbit appear mediated by specific, high affinity, cytoplasmic and nuclear receptors, particularly in pulmonary type II cells (8, 9). In humans, glucocorticoids also stimulate fetal surfactant production (10), possibly via specific glucocorticoid receptors on type II pneumocytes (11).

Estrogens also directly alter fetal lung differentiation and maturation (12). In the presence of maximal stimulatory amounts of glucocorticoid, low doses of estrone and estradiol increase pulmonary surfactant production, while higher doses are inhibitory (2). Estradiol binds to fetal guinea pig (13) and adult rat lung cytosols (14), sedimenting as a 4S species (15). Human fetal and adult lung also bind estradiol (10, 16, 17), albeit with a slightly lower affinity.

Like estrogen, androgens bind to cytosols from fetal and adult rat lung (14, 18) with no apparent sex difference in androgen binding.

In addition to promoting surfactant production and cell growth, hormones also strikingly alter the ultrastructural morphology of human lung explants. Addition of cortisol, prolactin, and insulin in vitro induces the formation of lamellar bodies in type II pneumocytes (3).

These studies suggest that bronchogenic carcinomas arising from hormone-responsive cells could retain, and perhaps magnify, some of the characteristics of the hormone-responsive state.

One potential mechanism for the action of steroids on primary bronchogenic carcinoma is receptor mediation. Receptors for estrogen (16, 19, 20), androgen (16, 20), and progesterone (16, 21) are present in histologically diverse primary and metastatic bronchogenic carcinomas. Tumor receptor incidence is variable, however, and may be dependent on either the histogenetic origin of the lesion or method of assay. To date, no systematic attempt has been made to characterize steroid binding in normal lung and primary bronchogenic carcinoma cytosols in a large series of patients under different assay conditions. The present study reports that a significant incidence of specific, high affinity receptors for androgen, estrogen, and glucocorticoid is present in normal and bronchogenic carcinoma cytosols.

MATERIALS AND METHODS

Materials. [1,2-3H]DEX3 (20.8 Ci/mmol), [2,4,6,7-3H]estradiol (96 Ci/mmol), [3H]R5020 (promogestone), a synthetic progestin (70-87 Ci/mmol), and [3H]R1881, a synthetic androgen (70-87 Ci/mmol) were purchased from New England Nuclear (Cambridge, MA) and repurified prior to use. Radioinert steroids were purchased from New England Nuclear and Sigma Chemical Co. (St. Louis, MO). Trizma base, EDTA, HAP, molybdc acid sodium salt, crystalline bovine serum albumin, a-monothioglycerol, dihydrothioitol, glycerol, phospholipase A, DNase, RNase, and trypsin were also purchased from Sigma. Activated charcoal, ammonium sulfate, and additional reagents were purchased from J. T. Baker Co. (Phillipsburg, NJ), and RNase-free sucrose was from ___

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Schwartz-Mann (Orangeburg, NY). Dextran T-70 was obtained from Pharmacia (Piscataway, NJ). ACS scintillation cocktail was from Amer sham-Searle Corp. (Arlington Heights, IL).

Methods (Tumor and Normal Lung Specimens). Fifty-five lung cancer tissue specimens were obtained from 55 adult patients undergoing primary surgery for lung cancer; 52 were obtained from male patients and 3 from female patients. Thirty (56%) were squamous cell carcinomas, 21 (38%) were adenocarcinomas, and 4 (7%) were anaplastic large cell tumors. All tumors were verified histologically and staged using the criteria established by the American Joint Committee for Cancer Staging (22). Twenty-seven specimens of histologically normal ventilating lung were recovered from 30 of the patients with squamous cell lung cancer, 17 of the 20 patients with adenocarcinoma and all of the patients with large cell anaplastic carcinomas.

All specimens were obtained during surgery when a portion of the specimen was sent for histopathological diagnosis (light and electron microscopic examination). The remaining tumor was trimmed of any necrotic tissue on ice, washed with ice-cold saline, blotted on sterile gauze, and immediately frozen in liquid nitrogen. Normal lung specimens were verified histologically free of tumor prior to receptor assay. Total elapsed time from removal of specimen to freezing was less than 15 min. Receptor analysis was either performed immediately or within 1 wk following surgery. Storage during this time was at −80°C.

Receptor Analysis (DCC). Tumor and normal specimens were initially analyzed for cytosol receptor for ER, PGR, AR, and GR by the standard DCC technique as previously reported (16). Specimens for assay were weighed while frozen and powdered in a tissue pulverizer (Thermomac Industries, Copiague, NY) at liquid nitrogen temperatures. All subsequent steps were performed at 0−4°C. The powdered tissue was homogenized 1:6 (w/v) with two 10-s bursts in a P-10 homogenizer (Brinkman Instruments, Westbury, NY) separated by a 30-s cooling period in the following buffer systems: estrogen, 10 mM Tris-HCl:5 mM EDTA:1 mM DTT (pH 7.4); androgen and glucocorticoid, 20 mM Tris-HCl:5 mM EDTA:12 mM α-monooctiglycerol:10% (v/v) glycerol with or without 20 mM sodium molybdate (pH 7.4); progestosterone, 10 mM Tris-HCl:5 mM EDTA:10 mM α-monooctiglycerol:10% (v/v) glycerol with or without 20 mM sodium molybdate (pH 7.4). The homogenate was centrifuged at 105,000 × g for 1 h. The supernatant solutions (cytosols) were removed, and the pellets were washed with an appropriate buffer (1:4, w/v). Following centrifugation for 1 h at 105,000 × g all the supernatant fluids (cytosols) were removed, and the pellets were discorcedated. Cytosol aliquots were incubated for 4 h (AR, ER) or 20 h (GR) at 0−4°C with 5 mM [3H]estradiol, 10 mM [3H]R1881, or 20 mM [3H]DEX with or without a 100-fold excess of radioiodinated estrone. Unbound estradiol was removed by incubation with a 1-ml DCC pellet for 15 min at 4°C and centrifuged at 3000 × g for 15 min. A 250-μl aliquot was layered on top of a 4.7-ml 10−30% sucrose gradient and centrifuged at 250,000 × g in a SS50.1 rotor for 16 h. Additional gradients for AR and GR incorporated 0.4 mM KC1 or 20 mM sodium molybdate. methyl-14C-labeled bovine serum albumin was used as a 4.6S marker according to the method of Martin and Ames (29). Each tube was punctured, and 135-μl fractions were collected under a constant pressure head and counted in 6 ml of ACS cocktail.

Specificity and Stability Analysis. Specificity of binding for androgen and glucocorticoid was determined by incubating competing radioiodinated ligands with 10−8 M [3H]R1881 and 10−9 M [3H]DEX in cytosols from normal lung for 20 h at 4°C and separating bound from free ligand with DCC. The degree of competition is expressed as percentage of bound [3H]-ligand with the binding of [3H]-ligand in the absence of competitor taken as 100%. Concentrations of radioiodinated competing ligand ranged from 10−8 to 10−1 M. Receptor stability in the presence of phospholipase A, DNase, RNase, trypsin, and (NH4)2SO4 (40% saturation) was determined in the presence and absence of 20 mM molybdate ion. Additional stability studies were carried out at 30°C and 37°C in the presence of 20 mM molybdate ion.

Statistical Analysis. A value over 3 fmol/mg of cytosol protein was determined by Scatchard analysis was arbitrarily taken as positive for tumor specimens in our earlier studies (16). No arbitrary cutoff was established for receptor binding to tumor or normal lung cytosols in the current study. The difference in receptor incidence of any particular class of receptor among histological groups was analyzed by Fisher's exact test (30). Receptor content and affinity among groups were analyzed by either analysis of variance for multiple group comparisons or simple t test (30).

RESULTS

Receptor for Estrogen and Progesterone. [3H]Estradiol bound to approximately 30% of normal lung cytosols assayed when HAP was used to separate bound from free ligand (Table 1). ER incidence, content, and affinity (Kd) were similar in squamous cell cancer cytosols when assayed by either DCC following ammonium sulfate precipitation, DCC alone (data not shown), or HAP in the presence of 20 mM molybdate ion (Table 1). In spite of the limited binding capacity, receptor affinity was generally lower than 1 nM. There was no suggestion of multiple binding sites on Scatchard analysis when either DCC or HAP was used to separate bound from free ligand or when radioiodinated DES was used as a competitive ligand. A similar ER profile was found in adenocarcinoma cytosols assayed by HAP. When present, binding was resistant to phospholipase A, DNase, and RNase but sensitive to trypsin and maximal at 4 h at 4°C in the presence of 5 mM [3H]estradiol (data not shown). ER prepared from a squamous cell carcinoma cytosol sedimented at 7−8S on a 10−30% low salt sucrose gradient (Chart 1), suggesting the binding...
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**Table 1**

Incidence of ER and PGR in normal lung and bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>ER Affinity $K_a$ (nM)</th>
<th>ER Content (fmol/mg protein)</th>
<th>PGR Affinity $K_d$ (nM)</th>
<th>PGR Content (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.9 ± 0.2</td>
<td>2.1 ± 1.1</td>
<td>0.6 ± 0.2</td>
<td>6.4 ± 3.6</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>1.8 ± 0.8</td>
<td>5.6 ± 3.3</td>
<td>3.9</td>
<td>16.6</td>
</tr>
<tr>
<td>DCC</td>
<td>2.8 ± 0.5</td>
<td>5.2 ± 1.7</td>
<td>1/7 (14)</td>
<td>8.3</td>
</tr>
<tr>
<td>HAP</td>
<td>2.8 ± 0.8</td>
<td>4.8 ± 3.7</td>
<td></td>
<td></td>
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</tbody>
</table>

Histopathology

<table>
<thead>
<tr>
<th>Normal lung</th>
<th>GR Affinity $K_a$ (nM)</th>
<th>GR Content (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCC</td>
<td>7.7 ± 1.3</td>
<td>45.9 ± 9.9</td>
</tr>
<tr>
<td>HAP</td>
<td>15.0 ± 1.6</td>
<td>51.4 ± 6.1</td>
</tr>
</tbody>
</table>

**Table 2**

Incidence of GR in normal lung and bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>GR Affinity $K_a$ (nM)</th>
<th>GR Content (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lung</td>
<td>7.7 ± 1.3</td>
<td>45.9 ± 9.9</td>
</tr>
<tr>
<td>DCC</td>
<td>13.4 ± 2.6</td>
<td>84.9 ± 18.8</td>
</tr>
<tr>
<td>HAP</td>
<td>4.9 ± 0.9</td>
<td>50.3 ± 10.0</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>4.9 ± 0.9</td>
<td>50.3 ± 10.0</td>
</tr>
<tr>
<td>DCC</td>
<td>10.8 ± 2.0</td>
<td>56.5 ± 13.5</td>
</tr>
<tr>
<td>HAP</td>
<td>10.6 ± 2.0</td>
<td>56.5 ± 13.5</td>
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</tbody>
</table>

Since initial studies demonstrated that GR content was increased an average of 10-fold [62.5 ± 33.9 fmol/mg of protein (SE) versus 6.8 ± 1.0; n = 4] in the presence of 20 mM molybdate, while slightly increasing apparent affinity ($K_a$ from 12.2 ± 1.8 nM versus 16.1 ± 6.7; n = 4), it was used in all subsequent assays reported here. Binding was resolvable into two distinct components using the DCC assay when assayed under equilibrium conditions. Approximately 30% of the cytosols assayed had an affinity for [3H]DEX significantly lower than 10 nM and a concomitantly higher receptor content (Table 2). Separation of bound from free ligand with HAP increased binding incidence (15 of 16; 94%). Only 2 of 15 (13%) cytosols had an affinity for [3H]DEX in a nM range, with the remainder in excess of 10 nM. Binding affinity was linear on individual Scatchard analysis and close to 1 on Hill analysis (suggesting a lack of cooperativity) with either DCC or HAP. Affinity ($K_a$) ranged from 0.5–9.0 nM in 15 of 21 GR-positive samples and 10.8–23.9 nM in 6 of 21 specimens, suggesting two distinct populations of receptor in the presence of molybdate, one with an affinity for ligand higher than 10 nM.

**Receptor for Glucocorticoid.** The incidence of high affinity binding of [3H]DEX to normal lung and bronchogenic carcinoma cytosols was significantly greater than estradiol or progesterone (Table 2). In the presence of 20 mM molybdate, [3H]DEX bound to 21 of 28 (75%) normal lung cytosols using the DCC assay. Protein was similar to that in other estrogen-responsive neoplastic tissues (31, 32) in spite of a reduction in affinity.

High affinity, low capacity binding for progesterone was also demonstrated in a limited series of normal lung cytosols in the presence of 20 mM molybdate using the DCC assay (Table 1). PGR incidence in tumor cytosols was negligible, which confirmed previous reports (16, 20) of a limited incidence of PGR in primary lung tumors.

**Receptor for Glucocorticoid.** The incidence of high affinity binding of [3H]DEX to normal lung and bronchogenic carcinoma cytosols was significantly greater than estradiol or progesterone (Table 2). In the presence of 20 mM molybdate, [3H]DEX bound to 21 of 28 (75%) normal lung cytosols using the DCC assay.
though apparent affinity was lower. In line with this observation, HAP did increase the ratio of specific to nonspecific binding in the presence of molybdate and was therefore used to separate bound from free ligand in specificity and saturability studies. There was virtually no contamination of the tissue specimens by blood proteins (as judged by hemoglobin content) due to prior thorough washing in ice-cold saline prior to freezing. GR binding in four cytosols prepared from histologically normal lung from noncancer patients was similar to that from normal lung cytosols obtained from patients with bronchogenic carcinoma (Kd, 4.7 ± 1.9 nM; 76.1 ± 34.5 fmol/mg of protein; range, 9.1–147.3; n = 4), suggesting that there was little, if any, indirect effect on GR in normal lung tissue specimens by the primary carcinoma.

Binding of [3H]DEX to normal lung (Chart 2) and bronchogenic carcinoma cytosols (data not shown) was specific for glucocorticoids, saturable (Chart 3), and trypsin sensitive. Maximal binding (fmol/mg of protein) was observed at 15 and 30 min at 37° and 30°C, respectively, with approximately 50% of apparent receptor binding degraded after 2 h at either temperature. At 4°C, binding of [3H]DEX did not plateau until approximately 20 h (Chart 3) but was saturable at 20–25 nm (data not shown) and may represent equilibrium between higher and lower affinity sites for glucocorticoid. Following a 20-h incubation with 20 nm [3H]-DEX, GR sedimented at 9.8S in low salt 10–30% sucrose gradients, 11.2S in the presence of molybdate (Chart 4A), and 4S in the presence of 0.4 M KCl (Chart 4B) when molybdate was omitted from the incubation buffer and gradient. GR was also present in 17 of 22 (77%) squamous cell carcinoma cytosols assayed by DCC with a mean binding affinity and content similar to those of normal lung cytosols assayed in the same manner (Table 2). An additional eight specimens assayed with HAP all expressed a similar binding profile. Ten of 12 (83%) adenocarcinoma cytosols bound [3H]DEX when assayed by DCC, while 8 of 8 bound [3H]DEX when assayed with HAP. There was no significant difference in GR affinity or content between methods or between control and tumor cytosols (Table 2). GR content was significantly higher in adenocarcinoma cytosols with an affinity for [3H]DEX > 10 nM as determined by DCC when compared to normal lung cytosols with the same affinity. This also appeared to be the case in four cytosols from large cell anaplastic lesions, although the number of these latter tumors was small (Table 2).

Receptor for Androgen. R1881 bound with high affinity but relatively low capacity to 22 of 24 (92%) normal lung cytosols assayed by DCC (Table 3) with minimal cross-reactivity from other steroids (Chart 5). A similar specificity profile was developed for squamous cell and adenocarcinoma cytosols (data not shown). Binding of [3H]R1881 was saturable at the temperature tested, and like GR, it reached maximal concentrations at 15 and 30 min at 37° and 30°C, respectively. Unlike GR, however, binding plateaued at 4 h at 4°C with 10 nm [3H]R1881 (Chart 6) and did not resolve into two groups with distinct affinities. Receptor for androgen sedimented at 7S in low salt gradients, 11S in the presence of 20 mM molybdate (Chart 7), and shifted to a lower molecular weight 4S species in 0.4 M KCl. [3H]R1881 also bound to a significant number of squamous cell (60%) and adenocarcinoma cytosols (87%) specifically and with high affinity (Table 3). In contrast to normal lung cytosols, the relative affinity of the putative receptor for androgen in squamous cell carcinoma cytosols in the presence of 20 mM molybdate was resolvable into two distinct groups when assayed by DCC. Seven of 12 (58%) of the squamous cell carcinoma cytosols binding [3H]-R1881 bound the ligand in a nanomolar range, while the remaining five (42%) expressed binding below 1 nM. Mean content was, as in the case of GR, inversely proportional to receptor affinity.
Each lesion exhibited a linear Scatchard plot indicative of a single class of binding sites. When HAP, rather than DCC, was used to separate bound from free ligand, binding affinity was <1 nM. The higher affinity (<1 nM) component of AR binding in squamous cell carcinoma cytosol using either DCC or HAP was similar to that in normal lung cytosols, but AR content was slightly lower.

Mean affinity for R1881 in adenocarcinoma was below 1 nM, similar to AR in cytosols from normal lung. R1881 also bound to two of four large cell anaplastic tumor cytosols (Table 3).

Tumor Grade (Stage of Disease). The differences observed in incidence, affinity, and content of GR and AR in bronchogenic carcinoma cytosols may be in part related to the degree of tumor differentiation. When receptor incidence was related to degree of tumor differentiation in squamous cell carcinoma, two of five (40%) AR-negative lesions were classified as Grade III-IV (poorly differentiated) with the remaining four showing a higher affinity but lower content (Table 4). This was not observed in adenocarcinoma where AR did not resolve into two distinct groups based on binding (Table 3). AR was not related to stage of disease in either squamous cell or adenocarcinoma. However, the majority of lesions in which AR was determined were T2, and further evaluation of the relationship between stage of disease and receptor content will have to await additional data.

GR incidence and affinity did not appear related to tumor grade in squamous cell carcinoma. Receptor content was lower in Grade III-IV than Grade I-II lesions, however. The small group size (n = 7) of poorly differentiated lesions ruled out a significant difference. This was not observed in adenocarcinomas where GR affinity and content were lower in well to moderately differentiated tumors (Grade I-II) (Table 4). Here again, the small group of Grade I-II lesions (n = 3) prevents a rigorous statistical assessment. GR content, but not affinity, also appeared related to stage of disease in squamous cell carcinoma. Staging was completed in 27 of 30 patients with squamous cell carcinoma. In 23 of 25 patients positive for GR in which staging was complete, there was a slight increase in mean GR content between Stage I (53.7 ± 13.7 fmol/mg of protein, n = 7) and Stage II (71.5 ±
RECEPTORS IN LUNG CANCER

Charts. Specificity of [3H]R1881 (1 nM) binding to normal lung cytosol in the presence of 20 mM MoO4. O, R1881; •, DHT; △, estradiol; ■, progesterone; ○, DEX; cortisol. HAP separation of bound from free ligand.

Chart 5. Specificity of [3H]R1881 (1 nM) binding to normal lung cytosol in the presence of 20 mM MoO4. O, R1881; •, DHT; △, estradiol; ■, progesterone; ○, DEX; cortisol. HAP separation of bound from free ligand.

5 6 16

TIME (hr)

Chart 6. Stability of AR in the presence of 10 nM [3H]R1881. At 4°C binding was saturable at 4 h and stable for 24 h. Receptor ligand dissociation was rapid at 37°C in the presence of 20 mM MoO4. HAP separation of bound from free ligand.

10.2 fmol/mg of protein, n = 5) and a sharp decline in Stage III lesions (32.8 ± 8.0 fmol/mg of protein, n = 11). When lesions of Stages I and II were grouped, the mean GR content was greater than in Stage III lesions (6.1 ± 9.2 fmol/mg of protein versus 32.8 ± 8.0, P < 0.05).

This was not observed in adenocarcinomas where there was an increase in mean GR content with stage of disease (Stage I, 53.1 ± 11.8 fmol/mg of protein, n = 6; Stage II, 84.2 ± 9.4 fmol/mg of protein, n = 3; Stage III, 115.8 ± 29.7 fmol/mg of protein, n = 5) in GR-positive lesions. The two AR-negative lesions were removed from a Stage I and Stage III patient. While the small number in each group prevents this increase from reaching statistical significance, it suggests that GR content of the primary tumor increases as the disease progresses. There was no relationship between stage of disease and the presence or absence of GR.

DISCUSSION

The present results suggest that a significant incidence of specific receptors for androgen, estrogen, and glucocorticoid with an affinity for hormone similar to those in other steroid-responsive tissues (8, 11, 13, 14, 17, 18, 23, 31-35) is present in normal adult human lung and bronchogenic carcinoma cytosols. The presence of a single class of high affinity (<1 nM), low capacity sites for estrogen in normal adult lung parenchyma confirms earlier observations of the ER in human (16, 19) and rat lung (14). Results from estrogen binding experiments using sucrose gradient analysis and HAP to separate bound ligand suggest that ER in adult human lung differs from that reported for fetal human and rabbit lung (10, 12). Scatchard analysis of ER binding in the presence of radioinert DES or estradiol suggests a similar binding profile. Fetal lung ER has a lower affinity for ligand, sediments at 4S, and in rabbits has no affinity for DES or ethynyl estradiol while avidly binding progesterone (10, 12). This suggests that a binding protein with a relatively high affinity for estrogens in addition to the classical estrogen receptor may be present in fetal lung. These observations also contrast with earlier reports of a negligible incidence of ER in a series of
squamous cell cytosols when DCC was used to separate bound from free ligand in the absence of molybdate ion (16, 20, 36) and suggest the importance of assay conditions when attempting to determine small amounts of receptor in the presence of significant amounts of nonspecific binding. In this context, Jones et al. (19) have reported an overall ER incidence of 55% in squamous cell and adenocarcinoma cytosols with binding characteristics close to the present study using Sephadex G-25 to separate bound from free ligand. The choice of an arbitrary cutoff in content as indicative of positive for receptor may also account for the reported differences in ER incidence (16, 36). The number of specimens available for assay in the present study was too small to draw any correlation between degree of histopathological differentiation and ER incidence or content. While a significant incidence of ER in adult lung parenchyma provides initial evidence that estrogens may continue to influence lung function in adults, additional studies will be necessary to better define the incidence and determine the functionality of this estrogen binding protein in adult human lung.

PRG incidence, while relatively high in normal lung, declined significantly in tumor cytosols confirming earlier observations (16, 20). There was no correlation between the presence of ER and PGR. These observations coupled with the lack of receptor in tumor cytosols and absence of relationship between putative progesterone binding and fetal lung function (12, 37) suggest a minor role for progesterone in lung homeostasis. Reports of displacable [3H]R5020 binding in fetal rabbit lung (18) may reflect a lower affinity, potentially less specific binding protein or a species difference. A larger series of cytosols will have to be assayed to resolve this question.

Androgen(s) clearly exhibit specific, high affinity, low capacity binding to normal adult human lung cytosols. While the presence of a cytosol receptor does not necessarily indicate a role for androgens in fetal lung development (12) and in the function of adult lung, the high incidence (93%) of AR and similarity to the binding characteristics reported for fetal and adult rabbit lung (18) and adult rat lung (14) suggest that this is a viable possibility (16). The presence of molybdate appears essential to reproducibly resolving the low capacity AR sites, particularly in tumor cytosols, as our own initial results (16) and those of others (20) omitting MoO₄ from the assay system showed a lower incidence of AR. AR in squamous cell carcinoma was present in two potentially distinct species based on receptor affinity using the DCC assay. Cytosols assayed at the same protein concentration (1–2 mg/ml) bound [3H]R1881 with an affinity either above or below 1 nM. This was not observed in a smaller series of cytosols when HAP was used to separate bound ligand.

Each Scatchard analysis gave a linear plot further suggesting two distinct species are observable when DCC is used to separate bound from free ligand in the presence of MoO₄. This is the first report of such a phenomenon in tumors of squamous cell origin, and as yet we have no definitive explanation for it. [3H]-DHT does, however, bind to normal skin squamous cell cytosols as two distinct species (38). Alternatively the expression of AR may be dependent on tumor grade or stage. Although AR affinity (content) did not appear related to tumor grade or stage of disease, our series was too small to statistically evaluate properly. It should be reiterated, however, that AR-negative lesions were either undifferentiated or poorly differentiated (Grades III–IV). Previous reports on AR in squamous cell lung cytosols show \( K_d \)s in a range of 0.1–1.0 nM (16, 20) with normal human lung less than 1 nM (16), similar to those in the present study. In contrast, AR affinity in adenocarcinoma cytosols exhibited only a single class of high affinity sites identical to that reported for AR in adenocarcinoma of the breast (31, 32).

GR was present in 82% of normal lung specimens assayed. Sucrose gradient analysis and specificity studies show the binding protein to be virtually identical with that reported for fetal and adult rabbit, rat, and human lung (4, 6, 8, 11, 35, 39). Our observations suggest that glucocorticoids may continue to influence lung function in adult human lung, possibly via receptor-mediated processes. When present in normal lung, GR was resolvable into two distinct binding moieties based on \( K_d \) when assayed in the presence of MoO₄ using DCC. This could be a function of the assay system as GR-positive specimens gave a linear Scatchard analysis with a \( K_d \) either above or below 10 nM. Alternatively this may indicate that functionally there is a differential sensitivity to endogenous or exogenous glucocorticoids in adult human lung. An analogous situation appears to be present in fetal rabbit lung. Pulmonary surfactant synthesis in fetal rabbit lung is sensitive to lower exogenous concentrations (10⁻¹⁰ M) of cortisol than cell growth (10⁻¹⁰ M) (5, 39) and additional protein synthesis (10⁻⁷ M) (6, 7), while cultured fetal rabbit lung binds glucocorticoid at roughly 10⁻⁸ M (9). Adult rat and rabbit lung cytosols have also been reported to bind glucocorticoid in a nanomolar range as have rabbit alveolar macrophages (8, 34). When the latter are activated by glucocorticoids, there is an increase in the number of binding sites (34). The slow saturation of GR at 4°C, absent in AR and ER binding but present in normal human fetal and neonatal cytosols (11), also suggests a multi-component binding system of potential physiological and pharmacological interest. Additional studies are needed to clarify this point.

Squamous cell carcinomas also show a significant incidence...
of binding. The incidence of binding in squamous cell carcinoma is significantly higher than previously reported (16, 21) using DCC in the absence of molybdate. Binding affinity (nm) was similar to that reported for GR in other neoplastic tissues (21, 31–33, 40). A single class of high affinity receptors for [3H]DEX was also present in essentially all primary adenocarcinomas of the lung assayed by either DCC or HAP. These results confirm our earlier observations (71%) (16) and suggest that GR content is greater in adenocarcinoma than normal lung cytosols when groups with similar binding constants are compared.

Demonstration of high affinity cytosol receptors for androgens and glucocorticoids in squamous cell and adenocarcinomas of the lung tempers speculation that these lesions may be manipulated by increasing or decreasing the level of glucocorticoid. Early studies suggest that administration of low doses of cortisone was associated with an apparent decrease in survival in patients with metastatic lung carcinomas (41). In contrast, dexamethasone has been reported to decrease the growth of alveolar cell carcinoma and Lewis lung carcinoma in vitro at low concentrations (40, 42). Tumor grade and stage of disease may also have to be taken into account in assessing the response of a particular histopathological grouping. We have presented suggestive evidence for a decrease in receptor content with increasing anaplasia and stage of disease in squamous cell carcinoma. However, GR content in adenocarcinoma appears to increase with dedifferentiation and tumor burden in adenocarcinoma.

Administration of testosterone propionate had no apparent effect on the progression of metastatic lung carcinoma (41) but did apparently relieve bone pain (44). Metabolism of this ester of the endogenous steroid must be taken into account in any estimate of its efficacy. The decrease in AR content coupled with an alteration in affinity in the more anaplastic squamous cell carcinomas suggest that only selected lesions in this histopathological category may respond to changes in circulating androgens.

The usefulness of ER, AR, and GR as an aid in determining patient prognosis and the appropriateness of steroid manipulation as future adjunct or palliative agents in bronchogenic carcinomas clearly remain to be determined. While previous reports suggest little chance of small (oat) cell carcinomas responding to steroids (16), the similarity in binding characteristics between bronchogenic carcinoma and other steroid-responsive tissues suggest a potential starting point in investigating whether steroids influence the natural history of bronchogenic carcinomas.

REFERENCES
