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Morphologic and biochemical changes in male rat lung after surgical and pharmacological castration

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Abstract

The morphology of the rat lung was studied by light microscopy in different situations: after surgical and pharmacological castration and after administration of testosterone to the castrated rat to determine if the androgen is required to maintain the normal morphology of the lung. We also determined the effect of flutamide on the phospholipid composition of both the surfactant and microsomes of the lung. Rats were separated into five groups: I - control non-castrated rats, II - castrated rats sacrificed 21 days after castration, III - castrated rats that received testosterone daily from day 2 to day 21 after castration, IV - castrated rats that received testosterone from day 15 to day 21 after castration, and V - control rats injected with flutamide for 7 days. The amount of different phospholipids in the surfactant and microsomes of the lung was measured in group I and V rats. At the light microscopy level, the surgical and pharmacological castration provoked alterations in the morphology of the lung, similar to that observed in human lung emphysema. The compositions of surfactant and microsomes of the lung were similar to those previously reported by us for the surgically castrated rats. These results indicate that androgens are necessary for the normal morphology as well as for some metabolic aspects of the lung.

Key words
- Lung
- Antiandrogens
- Phospholipids
- Flutamide
- Lung morphology

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Introduction

The alveolar surfaces of the lungs are lined with a lipid-protein complex called pulmonary surfactant. This material consists of approximately 90% lipids and 5-10% proteins. The surfactant component is synthesized by type II pneumocytes. The surfactant lipids spread as a monolayer at the air-liquid interface. Serious surfactant deficiency may compromise the structural and functional integrity of the alveoli and become life threatening. This situation may occur in the premature newborn (1). An abnormal surfactant also appears to be an important characteristic of the adult respiratory distress syndrome (2).

The alveoli that open into the alveolar sacs, the alveoli of the respiratory bronchioles, and the alveolar ducts make up the terminal lung unit (TLU). The surface covering of the surfactants is made up of a film of bubbles which occupies all the spaces of the TLU, giving it structural stability and regu-

lating the circulation of liquid throughout the TLU. The film of bubbles is not only formed on the alveolar epithelium and on the interalveolar septa but also across the openings of the alveoli, forming a network through which liquid channels connect the interstitial spaces, the surfactant and the surface liquids. The foam maintains the surface tension near zero, thus avoiding the collapse of the alveoli (3).

Normal rat lungs contain receptors for both androgens and estrogens, and the number of these receptors varies with age, sex and hormonal conditions of the animal (4). We have recently shown that 21 days after surgical castration, the phospholipid content in the adult male rat lung is increased compared to the non-castrated rats (5).

The effect of androgens on the target tissue can be studied not only by surgical but also by pharmacological castration, i.e., by administering antiandrogens such as flutamide (6,7). These substances recognize regions of the ligand-binding receptor domain that result in their dimerization and DNA binding, but leave the C-terminus of the ligand-binding domain in a form appropriate for protease and antibody recognition. As a result, the repressor function is not removed and the receptor is not able to induce transcription (8,9). The aim of the present study was to determine whether a) surgical castration produces morphologic changes in the lung, b) testosterone administration at different times can prevent the morphologic changes observed in the lung of castrated rats, and c) the antiandrogen flutamide administered to control rats produces the same morphologic and biochemical changes in the lung as those observed in the surgically castrated rat. In this way, we were able to obtain information about whether the androgen regulates some metabolic aspects that could alter the morphology of the lung. To our knowledge this is the first report that shows morphologic changes in the lung of androgen-deprived male rats.

Material and Methods

Chemicals

All reagents were of analytical grade. Phospholipid standards and flutamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals, feeding procedure

Four-month-old adult male Wistar rats weighing 250 g were kept in a light- (lights on 6:00-20:00 h) and temperature- (22-24°C) controlled room; rat chow (Cargill, Buenos Aires, Argentina) and tap water were available *ad libitum.* Rats were housed individually and divided into five groups. Group I was sham operated and used as control, group II was subjected to castration by simple orchidectomy, group III was subjected to orchidectomy and received testosterone (100 µg/kg body weight) daily from day 2 until day 21 after castration by intramuscular (*im*) injection, and group IV was subjected to orchidectomy and received testosterone daily from day 15 to day 21 after castration. Group V consisted of sham-operated animals which received flutamide *im* (5 mg/250 g body weight) dissolved in 5% ethanol and mixed with gelatin twice a day (at 8:00 a.m. and 8:00 p.m.) from day 15 to day 21 after surgery (10). All animals were killed 21 days after surgery.

Isolation of microsomes and extracelullar surfactant

Rats were anesthetized intraperitoneally (*ip*) with urethane (250 mg/100 g body weight in 1 ml of saline solution). The respiration of the rat was normal at all times prior to sacrifice. The trachea was cannulated and the lungs were filled with 2.5 ml ice-cold 0.9% saline solution. Lavages containing the extracellular surfactant were then collected. This procedure was repeated nine times with

ice-cold 0.9% saline. The combined lavages were centrifuged at 4ºC and 580 *g* for 10 min to sediment macrophages. The resulting supernatant was centrifuged at 198,000 *g* for 30 min in a Beckman ultracentrifuge LS 65 B using a 65 Ty rotor to obtain the surfactant pellet. After extraction of the extracellular surfactant, the lungs were quickly removed, washed with ice-cold 0.9% saline solution, and weighed. They were then homogenized in 0.32 M sucrose (1 g lung tissue/6 ml). The crude homogenate was centrifuged at 1000 *g* for 5 min and the supernatant was filtered and centrifuged at 7700 *g* for 20 min. The microsomes in the supernatant were isolated as a pellet after centrifugation at 100,000 *g* for 1 h in 0.8 M sucrose/Tris/NaCl buffer, pH 7.4 (11).

Analysis of phospholipids

Lipids were extracted from microsomes and the extract was resuspended in 2:1 chloroform:methanol (v/v). Phospholipids were separated into component species by thin-layer chromatography using silica gel H plates and chloroform, methanol, and water at a ratio of 65:25:4 (v/v/v), respectively, as solvent (12). Exposing the plates to iodine vapors identified the location of individual phospholipids, which were scraped off and quantified (13). The position of each phospholipid was determined using the respective standard, and the results were expressed as the percentage of total phospholipid phosphorus.

Light microscopy - histological techniques

The lung was perfused with 0.9% saline solution and then with Bouin's liquid through the pulmonary artery. Subsequently, sections of lung tissue were cut, which were submerged in the same fixing liquid for 5 h. All sections were obtained from the same region of the lung for effective comparison. The samples were embedded in paraffin and contiguous 5-6-µm thick sections were taken using a Reichter-Jung H 40 microtome and stained with hematoxylin-eosin. The photomicrographs were taken with a Leitz Dialux microscope equipped with a Leica camera.

Statistical analysis

Results are reported as means ± standard deviation. Data were analyzed statistically by ANOVA and the Tukey test.

Results

Body and lung weights

Body weight gains were similar for surgically and pharmacologically castrated rats, but lower for both groups in comparison to controls. The lung weight of surgically and pharmacologically castrated rats was similar and was higher compared to control (Table 1). When testosterone was administered to castrated rats the lung weight was similar to that of the control, as previously reported by us (5).

Analysis of phospholipids

The phospholipid content of microsomes of rats that received flutamide was increased compared to that of sham-operated controls (0.92 ± 0.2 and 0.14 ± 0.05 µmol/g wet tissue, respectively, $P<0.001$).

Table 1 - Body and lung weights of rats after surgical and pharmacological castration.

Data are reported as means ± SD, N = 8 for each case. Across a row, values with different superscript letters indicate significant differences by analysis of variance and the Tukey test. [a,b,c,d]$P<0.01$.

	Control	Surgical castration	Pharmacological castration
Body weight (g)			
Initial	268 ± 12	272 ± 9	285 ± 15
Final	312 ± 18	293 ± 7	305 ± 8
Gain	40 ± 12[a]	18 ± 5[b]	16 ± 7[b]
Lung weight (g)	1.57 ± 0.6[c]	1.89 ± 0.3[d]	1.92 ± 0.5[d]

Table 2 - Phospholipid composition of microsomes and extracellular surfactant from male rat lung after administration of flutamide.

Co: Control; F: control + flutamide; LPC: lysophosphatidylcholine; Sph: sphingomyelin; PC: phosphatidylcholine; PI + PS: phosphatidylinositol + phosphatidylserine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol. Data are reported as means ± SD for 8 rats in each group, expressed as percent of total phospholipids. [a,b]P<0.001, [c,d]P<0.01 by analysis of variance and the Tukey test.

	Microsomes		Extracellular surfactant	
	Co	F	Co	F
LPC	1.65 ± 0.1[a]	0.82 ± 0.08[b]	0.42 ± 0.01[a]	0.80 ± 0.02[a]
Sph	8.90 ± 0.9	8.74 ± 1.1	0.89 ± 0.05[c]	1.14 ± 0.10[d]
PC	67.50 ± 2.3[a]	76.09 ± 4.2[b]	78.70 ± 4.40[a]	68.70 ± 3.20[b]
PI + PS	10.7 ± 0.3[a]	8.24 ± 0.3[b]	2.80 ± 0.20[a]	10.50 ± 2.70[b]
PE	9.80 ± 0.0[a]	4.09 ± 1.6[b]	6.20 ± 0.60	5.40 ± 1.10
PG	3.2 ± 0.2	3.71 ± 0.5	9.70 ± 0.10[a]	14.20 ± 2.90[b]

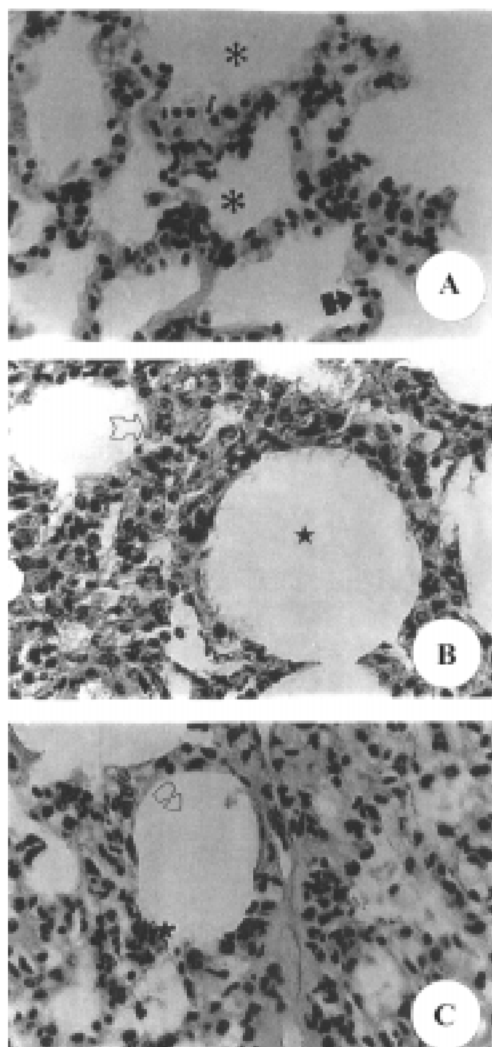

Figure 1 - Effect of surgical and pharmacological castration on male rat lung. H-E staining, X200. A, Control rat lung. The lung tissue appears normal and the alveoli are homogeneously distributed (*). The interalveolar septa (arrow) show typical development of the normal lung. B, Surgically castrated rat lung. The pulmonary parenchyma presents numerous large spaces caused by the rupture of the interalveolar septa (*). In other regions the fibrous connective tissue has increased and large numbers of macrophages are observed (arrow). C, Rat lung after flutamide administration. The parenchyma is altered and some zones have fibrous tissue that is invaded by macrophages (*). Large empty spaces can be seen (arrow) similar to those observed in castrated animals.

On a percentage basis, lysophosphatidylcholine (LPC), phosphatidylinositol + phosphatidylserine (PI + PS) and phosphatidylethanolamine (PE) were decreased and phosphatidylcholine (PC) was increased, but the amounts of sphingomyelin (Sph) and phosphatidylglycerol (PG) were unchanged in rats that received flutamide compared to sham-operated controls. In the extracellular surfactant of flutamide-treated rats the concentration of phospholipids increased in comparison with that of the control (21.82 ± 2.5 and 14.89 ± 1.2 µmol/g wet tissue, respectively, P<0.01). On a percentage basis, LPC, Sph, PI + PS and PG were increased, PC was decreased and PE showed no change compared to sham-operated control rats (Table 2).

Light microscopy

The lung parenchyma of a control rat is shown in Figure 1A. Significant morphological changes in lung parenchyma were observed in surgically castrated rats compared to controls (Figure 1B).

The TLU showed a significantly altered appearance. We observed large spaces formed by the fusion of the alveolar cavities as the interalveolar septa disappeared. The alveolar epithelium was not structurally affected, the pneumocytes appeared normal, and the alveolar surface was continuous. The interstitial tissue exhibited an evident hypertrophy due to the increase in the number of connective fibers and macrophagic invasion. The interstitial spaces contained an exceptionally large number of blood cells.

Significant damage to the lung parenchyma was observed in rats injected with flutamide. The alveolar structure was significantly altered, the thin histological weft disappeared and large spaces were formed by the collapse of the interalveolar septa. In the other regions of the lung, the amount of fibrous connective tissue increased significantly and large numbers of macrophag-

es invaded these zones compared to controls (Figure 1C). The lungs of castrated rats that received testosterone on day 2 did not show morphological changes (Figure 2A). On the other hand, when androgen was administered on day 15 the lung showed the same structural alterations as in the castrated rat lung. This indicates that testosterone did not reverse the effect of castration when administered at an advanced stage of lung damage (Figure 2B).

Discussion

The present results showed that pharmacological castration by the administration of flutamide modifies the composition of the phospholipids in the microsomes and in the extracellular surfactant of the male rat lung. These changes are similar to those previously observed in our laboratory using surgically castrated rats (14). Androgen suppression by surgical or pharmacological methods provokes significant alterations in the lung. These degenerative changes may be related, at least in part, to alterations in the chemical composition of cell membranes and lung surfactants. The alterations observed could be a consequence of the alteration in the composition of the phospholipids that make up the alveolar surface. This would provoke a disorganization of the gas-liquid interface with consequent damage to the film of bubbles, which keeps the surface tension near zero, thus allowing the TLU to maintain its physical integrity. The loss of the bubble film affects the network, primarily the zone corresponding to the alveolar septa, in which the amount of interstitial tissue is diminished, thereby providing little structural support. We observed that the septa break up and contiguous alveolar cavities fuse into one. The damage incurred cannot be due to the histological techniques used, since the same technique was employed in all cases.

The action of androgens on the lung has been studied primarily in the fetal lung. The synthesis of surfactant glycerophospholipids and proteins is under multifactorial control and is regulated by a number of hormones and factors, including glucocorticoids, prolactin, insulin, growth factors, estrogens, androgens, thyroid hormones and catecholamines acting through beta-adrenergic receptors, and cAMP (15).

Dihydrotestosterone (DHT) inhibits pulmonary surfactant production in fetal rabbits, and flutamide administration to pregnant rabbits eliminates the sex difference in the saturated phosphatidylcholine/sphingosine ratio in lung lavage by increasing the male ratios up to that observed in the females (16-18). Many observations support the view that surfactant deficiency is indeed the major cause of neonatal respiratory distress syndrome (19).

Male sex hormone (androgen) has an inhibitory effect on antenatal lung development. Androgen would decrease antenatal lung glucocorticoid receptor (GR). Furthermore, antenatal administration of DHT reduced tissue levels of GR mRNA and protein, consistent with androgenic inhibition

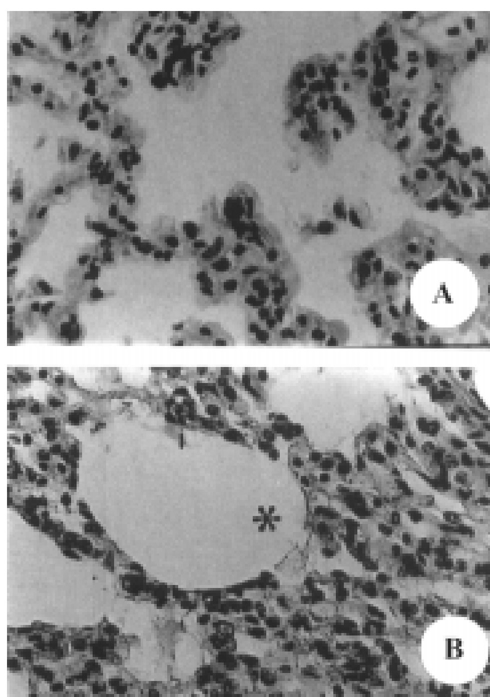

Figure 2 - Effect of testosterone on lung from a surgically castrated rat. A, Lung from a castrated rat that received testosterone on the second day after castration. The picture is similar to that of control rat lung (Figure 1A). B, Lung from a castrated rat that received testosterone 15 days after castration. The parenchyma is significantly damaged. Lysis of alveolar septa is observed. Large non-functional spaces are probably present (*).

of antenatal lung development by decreasing GR. Glucocorticoids and androgens exert opposite effects on fetal lung GR (20).

When explanted midgestation human fetal lung tissue is maintained in serum-free medium in the presence of dexamethasone, increased synthesis of saturated phosphatidylcholine (SPC) occurs. Addition of an equimolar concentration of DHT to the medium delays the spontaneous and dexamethasone-stimulated increases in SPC synthesis by 24 h after exposure. The antiandrogen flutamide neutralizes the effect of DHT, indicating that it acts through the androgen receptor to block the glucocorticoid (21). On the other hand, it is known that rats with pulmonary fibrosis present a decrease in the concentration of plasma corticosterone and testosterone and a lower content of pulmonary cytosol corticosteroid, androgen and estrogen receptors (22).

In the present study, in animals injected with flutamide, the parenchyma was enlarged by connective fibers. This could prevent the rupture of the alveolar structure, permitting the synthesis of cicatricial tissue. Contrary to what has been observed in castrated rats, blood in the alveolar space and lung parenchyma was absent. The morphology of the lung of castrated rats which were treated with testosterone on the second day after castration was similar to that observed in the control rat lung, possibly due to the fact that serum androgen concentration was similar to control (data not shown). When the cellular damage is produced 15 days after castration, the administration of testosterone does not reverse the structural alterations observed after castration, although the biochemical changes are reversed (14).

Our data demonstrate that the phospholipid composition of microsomes and extracellular surfactant in the male rat lung is highly dependent on androgenic stimulation and that the damage in lung parenchyma takes place in the absence of testosterone. Our data suggest that the lung should be regarded as an organ, which can be affected by gonadal steroid imbalances.

References

1. Jobe A (1988). The role of surfactant in neonatal adaptation. Perinatology, 12: 123-133.
2. Hallman M, Spragg R, Harrell JH & Moser KM (1982). Evidence of lung surfactant abnormality in respiratory failure. Journal of Clinical Investigation, 70: 673-683.
3. Scarpelli EM (1998). The alveolar surface network. A new anatomy and its physiological significance. Anatomical Record, 251: 491-527.
4. Morishige E & Uetake CA (1978). Receptors for androgen and estrogen in the rat lung. Endocrinology, 102: 1827-1837.
5. Ojeda MS, Gomez NN & Gimenez MS (1997). Androgen regulation of lung lipids in the male rat. Lipids, 12: 57-62.
6. Simard J, Luthy Y, Belanger A & Labrie F (1986). Characteristics of interaction of the antiandrogen Flutamide with androgen receptor in various target tissues. Molecular and Cellular Endocrinology, 44: 261-270.
7. Kallio PJ, Janne OA & Palvino JJ (1994). Agonist but not antagonists alter the conformation of the hormone-binding domain of the androgen receptor. Endocrinology, 134: 998-1001.
8. Tsai MJ & O'Malley BW (1994). Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annual Review of Genetics, 25: 89-123.
9. Mulder E & Kuil CW (1996). Deoxyribonucleic acid-binding ability of androgen receptors in whole cells: Implications for the action of androgens and antiandrogens. Endocrinology, 137: 1870-1877.
10. Sardanons ML, de las Heras MA, Calandra RS, Solano AR & Podesta EJ (1989). Effect of the antiandrogen flutamide on pituitary LH content and release. Neuroendocrinology, 50: 211-216.
11. Adachi H, Hayashi H, Sato H, Dempo K & Akino T (1989). Characterization of phospholipids accumulated in pulmonary surfactant compartments of rats intratracheally exposed to silica. Biochemical Journal, 262: 781-786.
12. Kuroki Y & Akino T (1991). Pulmonary surfactant protein A (SP-A) specially binds dipalmitoylphosphatidylcholine. Journal of Biological Chemistry, 266: 3068-3073.
13. Rouser G, Fluster S & Yamamoto A (1970). Two dimensional thin-layer chromatographic separation of polar lipid and determination of phospholipids analysis of spots. Lipids, 5: 494-496.
14. Ojeda MS (1995). Lipids of male rat lung. Effects of the androgens suppressed. Doctoral thesis, National University of San Luis, Argentina.
15. Mendelson CR & Boggaram V (1991). Hormonal control of the surfactant system in fetal lung. Annual Review of Physiology, 53: 415-440.
16. Nielsen HC, Zinman HM & Torday JS (1982). Dihydrotestosterone inhibits fetal rabbit pulmonary surfactant production. Journal of Clinical Investigation, 69: 611-

616.
17. Nielsen HC (1985). Androgen receptors influence the production of pulmonary surfactant in the testicular feminization mouse fetus. Journal of Clinical Investigation, 76: 177-181.
18. Klain JM & Nielsen HC (1993). Androgen regulation of epidermal growth factor receptor binding activity during fetal rabbit lung development. Journal of Clinical Investigation, 91: 425-431.
19. Jobe A & Ikegami M (1987). Surfactant for the treatment of respiratory distress syndrome. American Review of Respiratory Disease, 136: 1256-1275.
20. Sweezey NB, Ghibu F, Gagnon S, Schotman E & Hamid Q (1998). Glucocorticoid receptor mRNA and protein in fetal rat lung in vivo: modulation by glucocorticoid and androgen. American Journal of Physiology, 275: L103-L109.
21. Torday JS (1990). Androgens delay human fetal lung maturation in vitro. Endocrinology, 126: 3240-3244.
22. Tadzhiev FS (1991). Changes in plasma levels of corticosterone, testosterone and estradiol and their receptors in the lung cytosol in experimental fibrosing process in the lungs. Problemy Tuberkuleza, 3: 60-62.