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METABOLISM OF TESTOSTERONE IN THE ISOLATED PERFUSED RAT LUNGS

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SUMMARY

The metabolism of [4-^{14}C]-testosterone in the isolated perfused rat lungs was investigated following the administration of the substrate either *via* the pulmonary artery or *via* the trachea. After administration of testosterone in the circulating medium, 3.5% of the hormone was metabolized to various unconjugated metabolites during a single passage through the pulmonary circulation. It so seems that the lungs, receiving all the cardiac output, are one of the major sites of androgen catabolism in the rat organism. The major metabolites were 5α- and 5β-androstane-3α,17β-diols and various non-conjugated polar metabolites. After intratracheal instillation, testosterone was rapidly absorbed from rat lungs. Two minutes following instillation, 62% of the dose was recovered from the lungs. Two thirds of this was present as metabolites.

It is concluded that the lungs have an efficient metabolic capacity towards androgens. The availability of extractable substrate seems to be rate limiting for the pulmonary testosterone metabolism.

INTRODUCTION

We have earlier reported considerable testosterone metabolism by incubations of human, rat, rabbit, dog and cock lungs [1–7]. The presence of high levels of testosterone 5α-reductase in rat lung microsomal fraction has been confirmed by Verhoeven *et al.*[8].

There are several reports of the presence of corticosteroid 11β-hydroxysteroid dehydrogenase in mammalian pulmonary tissue *in vitro* [9–14], and also other corticosteroid metabolizing enzymes have been detected in dog lung [9–11]. This is also true for fetal lung tissue [15, 16]. Recently it has been suggested that the fetal lung belongs to glucocorticoid target tissues [17].

Thus far only one report on steroid metabolism in the isolated perfused lungs has appeared [18]. In this work, the presence of 11β-hydroxysteroid dehydrogenase activity in perfused rat lungs was confirmed by using either cortisone or cortisol as substrate. Guinea-pig lungs appeared to be much less active in this respect. The authors concluded that rat lungs should be regarded as a glucocorticoid activating tissue, as conversion of cortisone to other metabolites than cortisol was restricted.

Steroids used by inhalation route have recently been introduced in clinical use [19] which makes the metabolic fate of inhaled steroids an interesting topic of study. The rate of steroid absorption from rat lungs to the blood stream has been reported to correlate with the polarity of the substrate [20].

The present investigation on testosterone metabolism in the isolated perfused rat lungs was carried out to estimate the contribution of the lungs in the over-all testosterone metabolism in the rat. Furthermore, the metabolism and absorption of testerone following its instillation into isolated rat lungs *via* the trachea was investigated.

EXPERIMENTAL

The lungs were perfused by a modification of the method described by Hauge[21]. Male adult Wistar rats weighing 250–350 g were anesthetized with intraperitoneal phenobarbital (50 mg/kg). After tracheostomy the lungs were connected with a polyethylene tubing (i.d. 1.8 mm) to a pressure regulating respirator (Bird Mark 8, Bird Corp., California) and ventilation with air was begun (max. positive pressure 18 cm. H_2O). The thorax was opened, 500 IU of heparin was injected into the right ventricle and a glass cannula (i.d. 0.9 mm) was introduced into the pulmonary artery *via* the right ventricle. The left ventricle was excised, the pulmonary block removed and the cannula in the pulmonary artery connected to the external circulation. The interruption of the pulmonary circulation was less than 2 min.

The perfusion medium (65 ml) was fresh male rat blood slightly diluted with Tyrode solution [22] to give a hematocrit count of 0.28. The Tyrode solution contained 4 g/l of glucose. Additional experiments with this buffer solution as the only medium were performed to evaluate the effect of protein binding on the results. The medium was pumped (100 strokes/min) by a piston pump (Harvard Apparatus, Mass. model 1405) with a pulsatile flow of 10 ml/min. The perfusion pressure was maintained within 12–14/15–17 mmHg. The pH, pO_2 and pCO_2 of the medium were controlled with glass, calomel and membrane electrodes in a water jacketed flow chamber by a gas monitor (Radiometer, Copenhagen). To prevent alkalosis, the medium was gassed intermittently with 6%

CO_2 in air. The values in the medium during the perfusion were: pH 7.48 ± 0.06, pO_2 158 ± 13 and pCO_2 47.4 ± 5.9 (mean ± standard error of mean), reasonably near the physiological values of rat arterial blood [23].

Before connecting the lungs to the apparatus, the perfusion medium was circulated for 15 min in the thermostated circuit to warm it to 37°C and to mix the substrate with the medium. A magnetic stirrer was used in the reservoir. Excluding the silastic rubber tubing between different parts of the circuit and the piston, all surfaces in contact with the medium were of glass. The lungs were kept in a closed chamber to maintain the humidity. The ventilation was not interrupted during the manoeuvres.

Hemolysis was controlled by measuring plasma hemoglobin and found not to exceed 2% of total hemoglobin during 80 min of perfusion.

Loss of the steroid in the apparatus was checked from the radioactivity of the 5 ml samples in the recirculating experiment. A linear loss of radioactivity was seen, during 80 min of perfusion the radioactivity of the samples decreased 30%. Testosterone was present in the ethanolic washings of the apparatus in relatively greater amounts than in the medium of the corresponding experiment.

Amount of free steroid in the plasma was checked both by ultracentrifugation and by gel filtration with Sephadex G-25. The 4 h × 400,000 *g* supernatant of the medium contained 4% of the total radioactivity of the sample of 80 min perfusion. In gel filtration 6% of the radioactivity was eluted in the non-protein fractions of the samples taken at the beginning of the experiment or after perfusion for 80 min.

The cardiac output of anesthetized Wistar rats was measured by the electrical impedance technique (Impedancegraph 200 A, Finnomedical, Finland). The mean value was 204 ± 23 ml/kg/min (mean ± standard error of mean, $n = 7$).

Steroid analysis

As a substrate [4-^{14}C]-testosterone (S.A. 57,7 nCi/nmol, New England Nuclear Corporation) was used. It was purified by the t.l.c system on silicagel (see below). After that, the purity of the substrate was better than 98.5%. A dose of 0.4 nmol of the substrate was dissolved in 0.1 ml of 0.9% saline for instillation into rat trachea. In all other experiments a dose of 2.4 nmol of testosterone was mixed with the medium 15 min before the lungs were connected to the circulation.

The samples of the medium were taken in 50 ml of ethanol and the lungs were homogenized by an efficient homogenizer (Ultra-Turrax) in 50 ml of ethanol after washing them with 5 ml of Tyrode solution *via* the pulmonary artery. In non-recirculated perfusions, the sample sizes were 20 ml and 35 ml of the medium during the first 2 min and the next 3.5 min of perfusion, respectively. In the recirculating perfusions 5 ml samples from the reservoir were withdrawn with a syringe. The precipitate was removed by filtration through a 1 cm. thick layer of small glass beads and washed with 150 ml of ethanolic acetone (1:3) percolated through the same beads. The methods used for identification and quantitation of the metabolites are summarised in Table 1.

The column chromatography was carried out according to Nienstedt and Hartiala[24]. The free

Table 1. Summary of the identification and quantitation of the metabolites

1. *Extraction.* Sample taken in 50 ml of ethanol. Percolation with 150 ml of acetone–ethanol (3:1 v/v) over a 1 cm. thick layer of small glass beads in a column. Add octanol and evaporate other solvents, add hexane to make 40% for octanol.

2. *Column chromatography.* Eluate *lipids* with 40 ml of 4% octanol–hexane. Eluate octanol from the column with 20 ml of hexane and 80 ml of 0.25% ethanol–hexane. Eluate *free steroids* with 80 ml of methyl acetate and 20 ml of methyl acetate–water (96:4 v/v). Eluate *sulphates* with 15 ml of methanol–acetone (40:60 v/v). Eluate *glucuronides* with 15 ml of methanol–water (75:25 v/v). From each fraction a 10% sample is evaporated for liquid scintillation.

3. *Thin-layer chromatography.* All plates are run in two dimensions (10 cm. each):

A. Silica gel plates:
I: ethylene dichloride–methyl acetate (70:30 v/v)
II: hexane–hexanol (55:45 v/v)

B. Magnesium silicate–silica gel (9:1 v/v) plates:

a. To separate steroids with two keto- and/or hydroxyl groups:
I: ethylene dichloride–methyl acetate (90:10 v/v)
II: tert. amyl alcohol–hexane (10:90 v/v)

b. To separate steroids with at least three keto- and/or hydroxyl groups:
I: ethylene dichloride–methyl acetate (70:30 v/v)
II: tert. amyl alcohol–hexane (50:50 v/v)

4. *Radioautography.* t.l.c. Plates for 2–10 weeks on X-ray film.

5. *Quantitation.* Plate material bound and removed by using a colloidal material (Pharmacopea Fennica: Colloidium). Liquid scintillation.

steroid fractions were chromatographed and compared with added standards using the bidimensional "typical-atypical" t.l.c. systems developed by Nienstedt[25]. Most of the reference steroids used were obtained as generous gift from Professor W. Klyne and Dr. D. N. Kirk (Steroid Reference Collection, Westfield College, London) (see ref. 25 for details).

For the quantification of the metabolites the silica gel material of the chromatographic plates were wetted by a colloidal material (Pharmacopea Fennica: Colloidium), which hardens and binds the plate material as the dissolver (ether) evaporates. The areas of silica gel thus treated were then picked into the counting vials with pincers.

When tested with known amounts of ^{14}C labeled testosterone, progesterone and cortisol mixed in the same sample with 1.0 g of fresh rat lung, the recoveries of the three steroids after extraction, column chromatography and t.l.c. were: $72 \pm 2\%$ for testosterone, $72 \pm 2\%$ for progesterone and $71 \pm 2\%$ for cortisol (mean $\pm$ standard error of mean, $n = 4$). When the analysis was made without tissue in the mixture, the recoveries were $75 \pm 3\%$ for testosterone, $74 \pm 3\%$ for progesterone and $67 \pm 2\%$ for cortisol indicating a lower recovery of cortisol compared with the neutral steroids. This difference is probably caused by masking by tissual components of some high affinity binding site at the starting point of the absorbent layer.

To compensate for these methodical flaws, all results were compared with the analysis of the medium at the start of the corresponding experiments (Student's t-test). The following statistical symbols are used:

$<$ or $\times$: almost significant difference ($2P < 0.05$)
$<<$ or $\times\times$: significant difference ($2P < 0.01$)
$<<<$ or $\times\times\times$: highly significant difference ($2P < 0.001$)

RESULTS

Metabolism of testosterone in the medium (*Table* 2)

When the substrate was circulated in the apparatus with the medium but without the lungs, about 2% of testosterone was converted to its metabolites, mainly to androstenedione and polar metabolites. This metabolism was accomplished already during the first 20 min and no major changes were seen thereafter. When the medium was first circulated for 80 min through pulmonary circulation, after which the lungs were removed and testosterone added to the medium, more polar and dihydroxylated metabolites were formed than in the former case.

Metabolism of testosterone during a single passage through the lungs (*Table* 3)

The level of protein binding affects the pulmonary extraction and metabolism of testosterone. During the first 5.5 min of perfusion only negligible metabolite formation was seen, if the medium contained blood. On the contrary, considerable metabolism was detected in this time, when protein-free Tyrode solution was used as the sole medium. After perfusion, the amount of steroid bound by the lungs from Tyrode-medium was more than twice the amount taken up from blood-medium (Table 6).

Table 2. Metabolites of [4-^{14}C]-testosterone formed by diluted rat blood. A = medium + testosterone circulated in the apparatus for 80 min without contact with lungs. B = medium first circulated for 80 min through pulmonary circulation, after which the lungs were removed and testosterone added to the medium, which was thereafter circulated for additional 80 min in the apparatus. Percentages of total radioactivity, mean $\pm$ standard error of mean, $n = 4$ for A and $n = 3$ for B. The signs between the figures indicate statistical differences between the two systems, the asterisks below the values show statistical differences compared with the substrate

Substrate	20 min		40 min		60 min		80 min	
	A	B	A	B	A	B	A	B
T [1]	99±0.3	97±1 >	93±1	97±0.2	91±5	97±0.3 >	93±2	97±0.4 >	92±2
		x	xxx	xx	xx	xx	xxx	x	xxx
A [2]	0.0±0.0	0.6±0.4	0.1±0.1	1.1±0.4	0.2±0.2	0.9±0.4	0.7±0.4	0.9±0.3	0.7±0.4
		x		xxx		xx	xxx	xxx	xxx
M [3]	0.1±0.1	0.0±0.0	0.5±0.5	0.0±0.0	0.2±0.2	0.2±0.2	0.3±0.3	0.0±0.0	0.4±0.2
D [4]	0.1±0.1	0.2±0.0	1.0±1.0	0.3±0.1	2.4±2.1	0.7±0.1	1.6±0.2	0.7±0.3 <	1.8±0.3
			x		x	xxx	xxx	xx	xxx
P [5]	0.5±0.2	2.0±0.4 <	4.5±1.0	1.8±1.0	6.3±2.3	1.3±0.4 <<	4.5±1.5	1.3±0.3	4.8±1.7
		xx	xxx	xx	xxx		xxx	x	xxx

1) = testosterone
2) = androstenedione
3) = monohydroxymonoketosteroids
4) = dihydroxysteroids
5) = polar metabolites.

Table 3. Metabolites of [4-^{14}C]-testosterone formed during a single passage through isolated perfused rat lungs. Comparison of blood with protein-free medium. Percentages of total radioactivity, mean ± standard error of mean $n = 5$. The signs between the values express statistical differences between the two systems, the asterisks below the values indicate statistically significant differences compared with the substrate

Substrates		Time = 0-2 min		Time = 2-5 1/2 min		Lungs after persusion	
Blood	Tyrode	Blood	Tyrode	Blood	Tyrode	Blood	Tyrode
T[1] 94±1	96±1	94±1	91±2 x	92±1 >>	83±2 xxx	71±3 >	55±6 xxx
A[2] 2.5±0.7	0.4±0.3	2.5±1.1	0.6±0.4	3.5±1.2	2.1±0.1 xx	2.5±1.0	1.0±0.3 x
M[3] 0.3±0.2	0.6±0.2	0.4±0.2	2.3±1.6	0.5±0.1	1.1±0.6	3.0±0.4 xxx	5.5±1.7 x
D[4] 0.3±0.1	0.6±0.4	0.7±0.2	0.5±0.4	1.1±0.1 <<<	2.8±1.4	17±3 <	30±5
				xx		xxx	xxx
P[5] 3.2±1.2	2.9±0.4*	2.1±0.4 <<	5.1±0.7* x	2.6±0.3 <<<	11±1* xxx	5.5±1.4	8.9±1.1* xxx

1) = testosterone
2) = androstenedione
3) = monohydroxymonoketosteroids
4) = dihydroxysteroids
5) = polar metabolites
* Artificial binding of substrate in the application point (see experimental).

Metabolism of testosterone in recirculating perfusions (*Table* 4)

After 15.4 recirculations in 80 min, the percentage of testosterone in the medium had decreased 39.1 ± 2.1% (mean ± standard error of mean). Taking into account the binding of testosterone in the apparatus, the figure indicated that 0.75 nmol of testosterone was metabolized during these recirculations.

Both dihydroxy and polar metabolites were present in the medium in linearly increasing concentrations in the course of the perfusions. A small increase of monohydroxymonoketosteroids was also seen, while the share of androstenedione remained small. The percentage of the dihydroxy metabolites in the lungs was much larger than in medium after the perfusions, whereas the shares of testosterone and polar metabolites in the lungs were considerably smaller than in the perfusates.

Metabolism of testosterone after intratracheal instillation (*Table* 5)

After 0.4 nmol of testosterone in 0.1 ml of physiological saline was instilled intratracheally into perfused rat lungs, 38 ± 8% of the radioactivity was found in

Table 4. Metabolites of [4-^{14}C]-testosterone formed in recirculating perfusions of isolated rat lungs with blood-containing medium. The approximate number of recirculations is expressed under the corresponding perfusion time. Signs between the figures show statistical significancy of their differences and the asterisks below the values indicate statistical significancy of the difference of the corresponding value compared with the situation at the start of the perfusion. Percentages of total radioactivity, mean ± standard error of mean, $n = 5$.

0 min medium	20 min medium 3.3 passages	40 min medium 7.0 passages	60 min medium 11.0 passages	80 min medium 15.4 passages	lungs after perfusion
T[1] 95±1	86±3 x	80±1 >	70±3 >>	57±1 >>>	41±4 xxx
A[2] 1.0±0.3	2.7±1.2	1.8±0.6	1.4±0.7	1.5±0.8	1.3±0.6
M[3] 0	0.3±0.3	1.1±0.2 xxx	1.4±0.4 xx	3.4±2.6	4.7±0.9 xxx
D[4] 0.9±0.5	4.7±0.9 <<	11±2	16±4	22±4 <	41±4
	xx	xxx	xx	xx	xxx
P[5] 2.2±0.6	6.3±2.5	6.3±1.1 x	11±2 xx	20±5 xx	12±2 xx

1) = testosterone
2) = androstenedione
3) = monohydroxymonoketosteroids
4) = dihydroxysteroids
5) = polar metabolites.

Table 5. Metabolites of [4-^{14}C]-testosterone in the perfusate and the lungs 2 min after intratracheal instillation of the substrate. Percentages of total radioactivity mean ± standard error of mean, $n = 5$. The signs between the figures show statistically significant differences between the amounts of metabolites in the medium and lungs, the asterisks below the values indicate statistical difference compared with substrate

	Substrate	Medium		Lungs
T [1]	99±0.3	88±2	>>>	33±5
		xxx		xxx
A [2]	0.0±0.0	3.1±1.4		8.7±2.3
		xx		xxx
M [3]	0.2±0.1	1.1±0.3	<<	26±5
		xxx		xxx
D [4]	0.1±0.1	2.7±0.6	<<	21±4
		xxx		xxx
P [5]	0.6±0.2	4.8±0.9	<<<	10±1
		xxx		xxx

1) = testosterone
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3) = monohydroxymonoketosteroids
4) = dihydroxysteroids
5) = polar metabolites.

the perfusate 2 min after the instillation. Of this material 89% was testosterone and 11% its metabolites.

Only a third of the radioactivity in the lungs was present as testosterone 2 min after instillation. The percentages of all the main metabolite groups were larger in lungs than in the perfusate. A high percentage of 3α-hydroxy-5α-androstane-17-one was seen in the pulmonary tissue but not in the perfusate. This compound was not found among the main metabolites in the other experiments of this paper.

Pulmonary uptake of steroids in different experiments (Table 6)

The amount of radioactivity found in the lungs after one passage with the blood-medium did not increase with recirculations up to 15 times. The metabolism has proceeded much further in the latter case, but when the proportions of the pulmonary metabolites are compared with the percentages of these metabolites in the medium (Tables 3 and 4), no great variations in the tissue/medium differences are seen. If affinity of the steroid to the medium was diminished by using Tyrode medium, the pulmonary uptake was doubled.

Two minutes following intratracheal instillation of testosterone, the amount of unabsorbed steroid was equal to that taken up from the Tyrode medium in 5.5 min. Two thirds of the material was metabolized.

Individual metabolites from different sources

Besides androstenedione, the following metabolites of testosterone were identified:

The only identified *monohydroxymonoketo-steroid* was 3α-hydroxy-5α-androstan-17-one, which was present in pulmonary tissue 2 min after intratracheal instillation of testosterone. In continuous perfusions 17β-hydroxy-5α-androstan-3-one was tentatively identified in pulmonary tissue, where it formed less than 5% of the total amount of monohydroxymonoketosteroids. Both 5α- and 5β-androstane-3α,17β-diols were present among the *dihydroxylated* metabolites in different experiments. In all cases the amount of

Table 6. Steroids recovered from pulmonary tissue in different experiments. Percentage of total radioactivity, mean ± standard error of mean, $n = 5$. The signs between figures indicate statistical differences, the asterisks below the values show significant differences compared with instillation results

Perfusion type	Recirculated	Single passage		Single passage	Instillation
Medium	Diluted blood	Diluted blood		Tyrode solution	Diluted blood
Substrate (nmol)	2.4	2.4		2.4	0.4
Time (minutes)	80	5 1/2		5 1/2	2
Steroid in lungs (nmol)	0.10±0.01	0.10 ±0.02		0.22±0.04	0.23±0.05
T [1]	41±4	71±3	>	55±6	33±5
		xxx		x	
A [2]	1.3±0.6	2.5±1.0		1.0±0.3	8.7±2.3
	xxx	x		xx	
M [3]	4.7±0.9	3.0±0.4		5.5±1.7	26±5
	xx	xx		xx	
D [4]	41±4	17±3	<	30±5	21±4
	xxx				
P [5]	12±2	5.5±1.4		8.9±1.1	10±1
		x			

1) = testosterone
2) = androstenedione
3) = monohydroxymonoketosteroids
4) = dihydroxysteroids
5) = polar metabolites.

the 5α-epimer was at least four times that of the 5β-epimer. The only identified *polar metabolite* was 16α,17β-dihydroxy-4-androsten-3-one, which was identified in the medium of the recirculating perfusions. On the other hand, several other unidentified polar metabolites were also present as judged from their chromatographic behaviour [25].

No sulphate of glucuronide conjugation of the steroidal material was seen.

DISCUSSION

The present results on testosterone metabolism in the isolated perfused rat lungs are consistent with the earlier incubation experiments [1–8], which have pointed to considerable metabolic capacity towards testosterone in the lungs of various species, including man. Both the microsomal and the cytoplasmic portions of the cells, containing separate 5α- and 5β-reductases [1], seem to participate in androgen catabolism in perfused rat lungs. In accordance with the incubation results, no conjugation of the steroidal material was seen in the perfusion studies. The absence of UDP-glucuronyl transferase activity towards androgens in the lungs is in contrast to the considerable glucuronic acid conjugation of methylumbelliferone observed by us in similar perfusions of rat lungs [26, 27].

The lungs apparently take part in inactivating circulating testosterone, as the main metabolites formed by the perfused lungs have smaller androgenic activity than testosterone [28]. The coupling of the lungs to the circulation make the organ eminently suitable for controlling blood levels of circulating endogenous substances [29–32].

The disappearance of i.v. administered testosterone from rat circulation is rapid. From the data of Bruchovsky and Wilson[33], a half-life of about 4 min can be calculated. In our recirculating experiments, approximately 3.5% of the remaining testosterone was metabolized during each passage through the pulmonary circulation. As rat blood volume is one third of the cardiac output measured in this study or reported earlier [33, 34], the entire blood volume will be pumped three times in 1 min through the pulmonary circulation. If the results obtained reflect accurately the situation *in vivo*, the lungs should contribute with about 50% to the nonconjugative over-all catabolism of testosterone. Similarly, the amount if the hormone metabolized by the perfused rat lungs in this paper, is about 55% of the amount of the reported testosterone secretion by rat testis [35]. It is also reported that in man extraphepatic metabolism of testosterone accounts about 50% of its total metabolism [36, 37]. The nature of metabolites formed by eviscerated rats [33] do not exclude the possibility of their formation in the lungs.

A certain degree of caution is obviously indicated in the interpretation of these figures. Although the concentration of testosterone in the medium was near the plasma concentration of this hormone in man, it was 2 to 5 times the values reported for rat [38]. Consequently, as the availability of extractable substrate seems to be rate limiting in the pulmonary metabolism, the amount of metabolites formed of testosterone in these conditions may be larger than normally *in vivo*. The transit time of blood in the pulmonary circulation in the perfused lungs was longer than *in vivo*, as the flow was only one sixth of rat normal cardiac output [33, 34]. As it has been suggested that the distribution of steroid in different organs might be limited by the blood flow [39], the lengthened transit time might lead to increased extraction of testosterone in the perfused organ. Certain amount of the metabolism observed in the isolated perfused rat lungs could be due to enzymes released from the isolated perfused organ (Table 2).

The results of Bruchovsky and Wilson[33] of the slower disappearance of testosterone from the blood of castrated eviscerated rats point to about 20% extrasplanchnic metabolism of testosterone in rats, which is much less than in man [36, 37]. There are some possible causes for smaller extrahepatic metabolism of testosterone in rat compared with man. Rat blood does not contain high affinity testosterone binding globulin, which is present in human plasma [40]. As the binding of testosterone with high affinity ligands has been reported to protect the hormone from being extracted and metabolized in the liver [37], there exists a possibility that the lower affinity of testosterone binding in rat blood [28] might lead to increased extraction of testosterone in the rat organism. Also, hepatic blood flow during one minute in the rat is equal to rat blood vol. [33, 34] and consequently circulating hormones are transported more readily to this organ in the rat than in man.

Notwithstanding these comments it is apparent that the lungs are an important organ in testosterone catabolism. The rapid metabolism of either intratracheally administered testosterone or that administered i.v. in non-protein medium revealed a high pulmonary steroid metabolizing capacity. This may be important not only in the metabolism of endogenous testosterone but also of exogenous inhaled steroid. Such steroids have recently been introduced in clinical use [19], but no reports of their metabolism following intratracheal administration seem to be available. Our preliminary results with beclomethasone diproprionate [6] have pointed to considerable metabolism of the substrate following instillation into isolated perfused rat lungs.

In conclusion, the lungs seem to be one of the major organs of testosterone catabolism in the organism. The rapid metabolism of inhaled steroid may restrict the systemic side-effects of steroid inhalation therapy.

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