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THE EFFECTS OF CIGARETTE SMOKE EXPOSURE ON TESTOSTERONE METABOLISM IN THE ISOLATED PERFUSED RAT LUNG

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SUMMARY

Rats were exposed daily to cigarette smoke for 1 h for five consecutive days. The metabolism of [4-^{14}C]-testosterone by the isolated perfused lungs was studied on the sixth day. Slightly diluted rat blood, containing the radioactive substrate, was used as the perfusion medium. Compared to sham-exposure rats, the formation of reduced metabolites (dihydroxysteroids) was less and the metabolism of testosterone diminished after cigarette smoke exposure. After the perfusion the amount of polar non-conjugated metabolites was significantly higher in the smoke-exposed lung tissue than in the control lungs. No conjugate formation was observed.

The decrease in the formation of reduced metabolites seems to be due to an inhibition of the 4-ene-5α-reductase activity.

INTRODUCTION

The presence of several testosterone-metabolizing enzymes in the lungs of different species, including human lung, was demonstrated in our earlier reports using *in vitro* incubation techniques. The metabolism was shown to be quantitatively considerable when studied with an isolated perfused rat lung preparation (for ref. see 1). The presence of high levels of testosterone 5α-reductase in rat lung microsomal fraction incubations has also been reported by Verhoeven *et al.*[2].

Steroids and xenobiotics are catabolized by partially common mechanisms [3]. Cigarette smoke exposure increases the activity of pulmonary aryl hydrocarbon hydroxylase [4], which is a cytochrome P-448-linked monooxygenase. The present investigation was carried out mainly to study the possible effects of cigarette smoke exposure on the monooxygenase-dependent pulmonary metabolism of testosterone.

EXPERIMENTAL

Male adult Wistar rats weighing 270 to 320 g were used. The animals were exposed daily during 1 h to the smoke of ten filter cigarettes (1 mg nicotine and 16 mg tar per cigarette, as quoted by the manufacturer). The exposure was carried out during five consecutive days in an inhalation chamber as described earlier [4]. Control rats were exposed to air flow only. The perfusion experiments were made one day after the last exposure. The perfusion method has already been described in detail [1]. Briefly, the rats were anaesthetized with phenobarbital (50 mg/kg, i.p.) and after tracheostomy the lungs were connected to a pressure-regulated respirator and ventilated with air. After thoracostomy, heparin (500 IU) was injected into the right ventricle and the pulmonary artery was cannulated *via* the right ventricle. The left ventricle was removed and the cannula in the pulmonary artery was connected within 2 min to the perfusion apparatus. The perfusion medium consisted of rat blood, which was diluted to a hematocrit value of 0.28 with Tyrode solution containing 4 g/l glucose. The perfusions were made by a piston pump to generate a pulsatile flow rate of 10 ml/min with a pressure of 12–14/15–17 mmHg. The pH, pO_2 and pCO_2 of the medium were monitored and kept within the normal values of rat arterial blood by gassing with 6% CO_2 in air when necessary.

[4-^{14}C]-Testosterone (S.A. 57.7 Ci/mol, New England Nuclear Corp.), purified by t.l.c. [5], was used as substrate. A dose of 2.4 nmol of testosterone (in 2 ml of saline) was mixed with the circulating medium 15 min before the lungs were connected to the apparatus. Five-ml samples were withdrawn at 20-min intervals with a syringe and put immediately into 50 ml ethanol. The perfusions were continued for 80 min. The lungs were then homogenized in 50 ml ethanol with an Ultra-Turrax homogenizer.

The steroid analysis was based on Florisil column chromatography and bidimensional t.l.c. on silica gel with radioautography of the plates [5, 6]. Most of the reference steroids used were obtained as a generous gift from Professors W. Klyne and D. N. Kirk (Steroid Reference Collection, Westfield College, London). The results obtained with the smoke-exposed rat lungs were compared with those of the sham-exposed rats using Student's *t*-test for the statistical evaluations.

Table 1. Metabolites of [4-^{14}C]-testosterone in the perfusate at different perfusion times and in the pulmonary tissue after the perfusion

	0 Min		20 Min		40 Min		60 Min		80 Min		Pulmonary tissue 80 Min	
	Sham-exposed	Tobacco-exposed	Sham-exposed	Tobacco-exposed	Sham-exposed	Tobacco-exposed	Sham-exposed	Tobacco-exposed	Sham-exposed	Tobacco exposed	Sham-exposed	Tobacco-exposed
Testosterone	94 ± 2	95 ± 1	80 ± 2	86 ± 3	69 ≪ ± 2	80 ± 1	57 ≪ ± 3	73 ± 2	46 < ± 4	65 ± 3	26 ± 3	31 ± 2
Androstenedione	1.0 ± 0.3	1.0 ± 0.5	1.8 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	1.1 < ± 0.1	1.7 ± 0.2	0.9 ± 0.2	1.3 ± 0.1	1.1 ≪ ± 0.3	2.7 ± 0.1
Monohydroxymonoketosteroids	0.3 ± 0.3	0.1 ± 0.1	2.1 ± 0.4	1.1 ± 0.3	2.4 ± 0.6	1.1 ± 0.1	2.5 > ± 0.4	1.3 ± 0.2	2.5 > ± 0.3	1.1 ± 0.3	7.8 ± 2.2	7.6 ± 1.0
Dihydroxysteroids	1.1 ± 0.5	0.3 ± 0.1	9.4 ± 1.8	4.2 ± 1.5	18 > ± 3	8.3 ± 1.3	26 > ± 4	12 ± 1	33 > ± 5	17 ± 3	49 ± 4	34 ± 6
Polar (unconjugated) metabolites	3.8 ± 0.8	3.7 ± 0.8	6.3 ± 0.6	6.8 ± 1.3	9.5 ± 1.2	7.0 ± 1.4	13 ± 2	12 ± 1	16 ± 2	15 ± 1	15 < ± 1	25 ± 3

Values obtained with recirculating perfusions of isolated rat lungs with diluted blood for 80 min. Results given as percentages of the total radioactivity (mean values ± S.E.M.; $n = 4$). The sign > between two mean values indicates that they differ from each other at significance level $2P < 0.05$; the sign ≫ indicates a significance level of $2P < 0.01$ (Student's t-test).

RESULTS

After the perfusion, the mean radioactivity per animal found in the lungs corresponded to 0.46 nmol (±0.06; S.E.M.) testosterone in the smoke-exposed, and 0.40 nmol (±0.09; S.E.M.) in the sham-exposed series.

Table 1 gives the amounts of the metabolites present in the perfusion medium at different times, as well as those found in the lung tissue after the 80-min perfusion experiments. No sulphate or glucuronide conjugates of the steroidal material was detected.

No qualitative differences were observed in the metabolites formed in the two experimental series. 4-Androstene-3,17-dione was present in almost every sample but neither 5α-androstane-3,17-dione nor its 5β-epimer could be found with certainty. In the monoketomonohydroxysteroid area of the thin-layer plates, usually two metabolites were found. Their chromatographic mobilities corresponded to those of authentic 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone; usually the larger spot) and 3α-hydroxy-5α-androstan-17-one (androsterone). In the dihydroxysteroid area, usually three metabolites were found, their chromatographic mobilities corresponding, in decreasing order of spot size, to the following reference steroids: 5α-androstane-3α,17β-diol, 5β-androstane-3β,17β-diol and 5α-androstane-3β,17β-diol. The presence of small amounts of 5β-androstane-3α,17α-diol, 4-androstene-3α,17β-diol or 4-androstene-3α,17β-diol could not be excluded. The polar unconjugated metabolites contained up to about seven metabolites, of which only 6β,17β-dihydroxy-4-androsten-3-one and 16α,17β-dihydroxy-4-androsten-3-one were tentatively identified on the basis of their chromatographic mobilities in the bidimensional t.l.c.

The amount of the dihydroxylated metabolites in the perfusion medium decreased significantly after cigarette smoke exposure (Table 1). Visual comparison of the radioautographs suggested that this decrease affected mainly or exclusively the 5α-metabolites. This decrease was also reflected by the significant decrease in the consumption of the substrate. The trend was similar in the lung tissue, but here the differences could not be shown to be statistically significant. There were no significant differences in the amounts of the polar nonconjugated metabolites in the perfusion medium. However, significantly larger amounts of polar metabolites were found after the perfusion in the lungs of cigarette smoke-exposed rats.

DISCUSSION

Exposure to cigarette smoke has a marked influence on pulmonary testosterone metabolism in the rat. The main effect in the present experiment was, rather unexpectedly, the decrease in the metabolic clearance rate of testosterone by approximately 40%. This was due to diminished appearance of dihydroxysteroids and monohydroxymonoketosteroids into the perfusion medium.

There probably are several metabolic routes from testosterone to dihydroxysteroids in the lung tissue. The presence of 3α-hydroxy-5α-androstan-17-one as one of the monohydroxymonoketo metabolites suggests that one route might be as follows: testosterone $\xrightleftharpoons{17\beta\text{-dehydrogenase (A)}}$ androstenedione $\xrightarrow{4\text{-ene-}5\alpha\text{-reductase (B)}}$ 5α-androstanedione $\xrightarrow{3\text{-dehydrogenases (C)}}$ 3-hydroxy-5α-androstane-17-ones $\xrightarrow{17\beta\text{-dehydrogenase (D)}}$ 3-hydroxy-5α-androstane-17β-ols. Androstenedione and, especially, 5α-androstanedione must be rather short-lived as intermediates.

Another, more direct, route, starting with 4-ene-5α-reductase is suggested by the presence of 17β-hydroxy-5α-androstan-3-one as an intermediate. In both cases the site of the partial metabolic block could be at the reductase step (B). An inhibition at (C) or (D) should have led to the accumulation of 5α-androstanedione or monohydroxymonoketosteroids, respectively. A block at (A) is not probable as the amount of androstenedione was actually increased in the perfusate. The reversibility of the reaction (A) could explain why this increase was not larger.

The exact mechanism of the inhibition of 4-ene-5α-reductase activity remains open to discussion. It might, of course, be an expression of toxic effects of the cigarette smoke components. A serious generalized cellular damage would not seem very probable, however, as similarly or more heavily exposed rats showed remarkably increased activity of aryl hydrocarbon hydroxylase [4]. Cigarette smoke exposure increases the metabolism of benzo(a)pyrene in the isolated perfused rat lung several fold [7]. Benzo(a)pyrene is metabolized by aryl hydrocarbon hydroxylase. A parallel phenomenon is the temporary inhibition of epoxide hydrase in the lung tissue by cigarette smoke exposure [4].

In principle, any of the bifunctional steroid intermediates mentioned above could be transformed into trifunctional, tetrafunctional, etc., metabolites by enzymes (hydroxylases) which add new hydroxyl groups to various sites of the steroid skeleton. In the chromatographic system used, these metabolites migrate in the fraction of "polar" metabolites (Table 1). These hydroxylation reactions were, as expected, enhanced in the present study by cigarette smoke exposure. The effect was, however, demonstrable in the lung tissue samples only. No clear explanation can be given for the fact that these metabolites were not found in increased amounts in the perfusing medium, too. Earlier experiments [1] seem to point out that these polar metabolites should be removed from the lung tissue to the circulating fluid rather more rapidly than nonpolar compounds.

There are different forms of cytochrome P-450 in the liver, which catalyze testosterone hydroxylation at the 6β, 7α and 16α positions with different rates [8, 9]. Because the catalytic activities of these different

forms of cytochrome P-450 toward testosterone and benzo(a)pyrene are different [9], and because the effect of cigarette smoke on the different forms of cytochrome P-450 probably varies, the formation of hydroxylated metabolites of testosterone and benzo-(a)pyrene seems to have changed differently after cigarette smoke exposure. This offers a possible explanation to the unexpectedly small increase of hydroxylation of testosterone in this study.

The overall effect of cigarette smoke-exposure for testosterone catabolism is diminution rather than enhancement, even in the lung tissue samples. This sparing of the substrate was statistically significant, however, only in the perfusing fluid.

The present study clearly calls for further studies on the effects of smoking on the steroid metabolism. As the lung easily permits inhaled smoke constituents into the circulation, the potential enzyme inducers (e.g., aromatic hydrocarbons) and inhibitors (e.g., carbon monoxide) [10] have ready access to the different sites of both steroid synthesis and catabolism. It is interesting to note that ethanol drinking has been reported to cause lowered plasma testosterone concentrations, mainly as a result of increased hepatic 4-ene-5α-reductase activity [11]. The enhancement of hepatic steroid hydroxylation reactions by different inducing agents is well documented [3]. There also exists an isolated report on the *in vitro* induction of hepatic testosterone 4-ene-5α-reductase by phenobarbital [12], but *in vivo* results of drug effects on the metabolism of intravenously administered testosterone pointed to decreased 4-ene-5α-reductase activity [13]. Thus the over-all effects of exogenous compounds on steroid metabolism remain to be settled. The possible correlation between pulmonary steroid metabolism and lung cancer is beyond the scope of this study. However, it should be noted that urinary androgen excretion of lung cancer patients differs from that of normal subjects [14] and that there is a sex difference in steroid metabolism in the liver of the rat [15].

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