Reduced Production Rates of Testosterone and Dihydrotestosterone in Healthy Men Treated With Rosiglitazone

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The effect of the thiazolidinedione, rosiglitazone (8 mg/d for 7 days), on the production rates of testosterone (T), dihydrotestosterone (DHT), and cortisol (F) was studied in healthy men (n = 10) using the stable isotope dilution technique and mass spectrometry. Treatment with rosiglitazone resulted in a decrease in the production rates of T from, basal, 318 ± 62 μg/h to 272 ± 72 μg/h ($P < .05$). Production rates of DHT fell from, basal, 21 ± 6 μg/h to 17 ± 5 μg/h ($P < .05$). Hence, the ratio calcuated from the production rates of T and DHT was unchanged (basal, 17 ± 7; rosiglitazone, 17 ± 3). Production rates of cortisol were unchanged (basal, 577 ± 136 μg/h; rosiglitazone, 627 ± 141 μg/h). These results suggest that a clinically relevant dose of at least one thiazolidindione, rosiglitazone, impedes the production of testosterone in man.
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THIAZOLIDINEDIONES improve the sensitivity to insulin and thus glycemic control,[1] presumably by an agonist action on the nuclear peroxisome proliferator-activated receptor γ.[2] After having been initially aggressively promoted as the first agent of this class, troglitazone was withdrawn from the market due to hepatic side effects.[3] There is scarce information about other metabolic effects of this comparatively new group of antidiabetic drugs, but at least in regard to troglitazone evidence has been presented in vitro that they may interfere with steroidogenesis by influencing the activity of 17α-hydroxylase, 17,20-lyase, aromatase, and 3ß-hydroxysteroid-dehydrogenase.[4-7] In male obese Zucker rats troglitazone decreases plasma concentrations of dihydrotestosterone (DHT).[8] Using the stable isotope-labeled technique we have determined the endogenous production rates of cortisol (F), testosterone (T), and DHT to clarify whether a clinically relevant dose of a second-generation thiazolidinedione, rosiglitazone, would influence steroid hormone production in man.

MATERIALS AND METHODS

Experimental Protocol

Ten healthy, non-obese men aged 21 to 35 (25 ± 4) years who had been carefully informed about the aims and the possible risks of the study gave their written consent to participate. The study protocol was approved by the local ethics committee.

During the 24-hour period preceding each test the volunteers collected their complete urine for later analysis of urinary steroid excretion rates. On the day of the experiments an indwelling catheter was inserted at 8 AM into an antecubital vein. A blood sample was obtained for the determination of blood glucose and of the serum concentrations of luteinzing hormone (LH) and follicle-stimulating hormone (FSH). Each volunteer then received a continuous (Infusomat, Braun-Melsungen, Germany, 40 mL/h, t = 10 hours) intravenous infusion containing 2.0 mg 1α,2α-D-cortisol (CIL Isotopes, Andover, MA), 250 μg 1α,2α-D-testosterone (CIL Isotopes), and 250 μg 2,3,4-^{13}C-dihydro-testosterone (Steroko Chemicals, Vienna, Austria) in 500 mL of 0.9% saline also containing 2 mL of the individual's own blood. Samples of the infusate were obtained from the end of the infusion line at the beginning and at the end of each infusion, and actual infusion rates were corrected for losses by adsorption. After an equilibration period of 6 hours (at 2 PM) a second indwelling catheter was inserted into the contralateral arm and blood samples (5 mL) were obtained at 20-minute intervals for 4 hours (ie, until 6 PM). Blood samples were subsequently pooled for the whole period of 4 hours. These pooled samples were used for analysis by gas chromatography–mass spectrometry (GC/MS).

Two investigations were done in each volunteer in randomized sequence with an interval of 4 weeks: under basal conditions and following the treatment with 8 mg (4 mg twice daily) of rosiglitazone (Avandia, SmithKline Beecham, Brentford, MiddLesex, UK) for 7 days. No placebo was administered prior to the control experiment.

Materials

All organic solvents were of high-performanc liquid chromatography (HPLC) grade and purchased from Baker Chemicals, Phillipsburg, NJ. Nonactive F (11β,17,21-trihydroxy-4-pregnene-3-20-dione) was obtained from Sigma (St Louis, MO). Nonactive T (4-androsten-17β-ol-3-one) and DHT (4-androstan-17β-ol-3-one) were obtained from Steraloids (Wilton, NH). Radioactive (^{3}H)1,2,6,7-cortisol (specific activity, 60 Ci/mmol) was purchased from Amersham (Amersham, UK). Radioactive (^{3}H)1,2,6,7-testosterone (specific activity, 95 Ci/mmol) and radioactive (^{3}H)1,2,4,5,6,7-dihydrotestosterone (specific activity, 110 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Stable-labeled 1,2-D-cortisol (isotopic enrichment, 99.0%) and 1,2-D-testosterone (isotopic enrichment, 99.0%) were purchased from CIL. Stable-labeled 2,3,4-^{13}C-dihydro-testosterone (isotopic enrichment, 99.0%[9,10]) was obtained from Steroko Chemicals, Vienna, Austria.

GC/MS Analysis of Urinary Steroid Excretion Rates

Urine samples were processed[11] and analyzed by GC/MS[12] as reported previously.

Sample Preparation and Analysis by GC-MS

The sample preparation and the GC/MS analysis of F, T, and DHT has been described previously.[13-15] Production rates (PR) of F, T, and DHT and were calculated from the product of the known infusion rate (Rt) and the ratio of tracer infusate enrichment (Et) to tracer dilution in the plasma (Es): (PR = Rt × [Et/Es − 1]).[16]

Plasma concentrations of native (unlabeled) T, DHT, and F were determined as part of the GC-MS analysis. Plasma concentrations of LH and FSH were determined radioimmunologically by a commercially available kit.

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Table 1. Excretion Rates (mg/24 h) of Steroid Metabolites in Healthy Volunteers Under Basal Conditions and Following Treatment With Rosiglitazone 8 mg/d for 7 Days

	Basal	Rosiglitazone
Androsterone	2.1 ± 1.1	2.4 ± 1.4
Etiocholanolone	1.2 ± 0.8	1.3 ± 0.4
Pregnanetriol	0.5 ± 0.3	0.4 ± 0.2
Tetrahydrocortisone	0.9 ± 0.5	1.3 ± 0.7
Tetrahydrocortisol	0.4 ± 0.3	0.7 ± 0.3
Allo-tetrahydrocortisol	0.3 ± 0.2	0.2 ± 0.1
6β-hydroxy-cortisol	0.04 ± 0.02	0.05 ± 0.05
Sum of all steroids	6.9 ± 3.5	8.3 ± 2.0
Main glucocorticoids*	2.4 ± 1.0	1.6 ± 1.0
Ratio of cortisone/cortisol metabolites†	0.7 ± 0.2	1.1 ± 0.9

*Tetrahydrocortisone + allo-tetrahydrocortisone + tetrahydrocortisol + allo-tetrahydrocortisol.

†(Tetrahydrocortisone + allo-tetrahydrocortisone)/(tetrahydrocortisol + allo-tetrahydrocortisol).

Statistics

Data in the text in the tables are given as means ± SD. Student's *t* test for matched and unmatched pairs was used for statistical evaluation.

RESULTS

Concentrations of blood glucose were similar during basal conditions (84 ± 5 mg/dL) and after pretreatment with rosiglitazone (82 ± 5 mg/dL; $P > .05$) as were the serum concentrations of LH (basal, 3.8 ± 1.2 mU/mL; rosiglitazone, 4.9 ± 3.0 mU/mL; $P > .05$), of FSH (basal, 4.2 ± 2.1 mU/mL; rosiglitazone, 5.0 ± 2.8 mU/mL), and of F (basal, 7.5 ± 1.6 μg/dL; rosiglitazone, 7.1 ± 2.0 μg/dL; $P > .05$). Following treatment with rosiglitazone there was a small decrease in the serum concentrations of T (basal, 6.4 ± 1.4 ng/mL; rosiglitazone, 5.5 ± 1.0 ng/mL; $P < .05$) and a small albeit statistically significant rise in the serum concentrations of DHT (basal, 0.6 ± 0.2 ng/mL; rosiglitazone, 0.7 ± 0.2 ng/mL; $P < .05$).

Urinary Steroid Excretion Rates

The cumulative steroid excretion (sum of all estimated 21 steroid metabolites) was similar during basal conditions (6.9 ± 3.5 mg/24 h) and following rosiglitazone (8.3 ± 2.0 mg/24 h. Neither were there any qualitative changes in the pattern of steroid excretion rates (ie, altered ratios between the various metabolites, such as the ratio of the major cortisol/cortisone-metabolites) (Table 1) nor were there any quantitative changes in the excretion rates of 6β-hydroxycortisol or of the other investigated steroid metabolites, androsterone, etiocholanolone, pregnanetriol, tetrahydrocortisol, tetrahydrocortisone, allo-tetrahydrocortisol, and allo-tetrahydrocortisone.

Production Rates of Cortisol, Testosterone, and Dihydrotestosterone

The production rates (μg/h) of F remained unchanged, whereas those of both T and DHT decreased ($P < .05$) by approximately 15% to 20%. Thus, the ratio calculated from these 2 production rates remained unchanged following treatment with rosiglitazone (Table 2).

DISCUSSION

Thiazolidine derivatives constitute a relatively new group of insulin-senitizing agents believed to improve glycemic control by an agonist action on the nuclear peroxisome proliferator-activated receptor γ.[1, 2] In addition, thiazolidinediones have various other metabolic effects, including interference with the production and metabolism of various hormones. In humans troglitazone enhances the metabolism of oral contraceptives[17] and of the synthetic glucocorticoid dexamethasone[18] by an action on the hepatic enzyme CYP3A, and subsequently (after 20 days) increases the urinary excretion of 6β-hydroxycortisol.[19] In the present investigation we failed to detect any changes in the excretion rates of 6β-hydroxycortisol or of any other steroid metabolite following treatment with rosiglitazone. This could be due to the shorter duration of treatment (1, rather than 3 weeks[19]). Alternatively, the hepatic action of troglitazone and the ensuing potential therapeutic problems and/or advantages may not be shared by other members of of the thiazolidine group.[18]

Since the difference between the 2 main cortisol metabolites (tetrahydrocortisol and allo-tetrahydrocortisol) and the 2 main metabolites of cortisone (tetrahydrocortisone and allo-tetrahydrocortisone) is the hydroxy (*v* keto) group in position 11, the ratio between the former and the latter 2 metabolites may be used to globally assess the activity of 11β-hydroxysteroid-dehydrogenase. The results summarized in Table 1 show that the short-term administration of rosiglizatone does not influence 11β-hydroxysteroid-dehydrogenase in healthy, non-obese men, whereas a rosiglitazone-induced inhibition of 11β-hydroxysteroid dehydrogenase activity has recently been demonstrated in adipocytes.[20]

In vitro, thiazolidinediones interfere with steroidogenesis by influencing the activity of 17α-hydroxylase, 17,20-lyase, aromatase, and 3β-hydroxysteroid-dehydrogenase.[4-7] In male obese Zucker rats, troglitazone decreases plasma concentrations of DHT. Concentrations of T in these animals exhibit at least a tendency to decrease.[8] The results of the present investigation, obtained with the stable isotope-labeled technique, indicate that in a clinically relevant dose—albeit given for a comparatively short period of time—rosiglitazone does not influence the endogenous production rate of F. There is, however, a fall of endogenous production rate of T. In parallel, production rates of DHT are also reduced, which causes the

Table 2. Production Rates of Cortisol, Testosterone, and Dihydrotestosterone in 10 Healthy Men Under Basal Conditions and Following Treatment With Rosiglitazone 8 mg/d for 7 Days

	F (μg/h)	T (μg/h)	DHT (μg/h)	PR [T]/PR [DHT]
Basal	577 ± 136	318 ± 62	20 ± 6	16.9 ± 7.0
Rosiglitazone	627 ± 141	272 ± 72	16 ± 5	16.9 ± 2.8
P value	NS	<.05	<.05	NS

Abbreviations: PR, production rate (from 2 PM to 6 PM); NS, not significant.

ratio of the production rates of T/DHT to remain constant. Rather than for a rosiglitazone-induced impaired 5α-reductase activity, these results suggest that the decreased production of DHT could be secondary to impaired T production. Although the present investigation does not permit us to distinguish a pituitary from a peripheral effect of rosiglitazone the fact that this thiazolidinedione impairs steroid production in an in vitro model rather argues for a peripheral, ie, testicular, mode of action. Thus a clinically relevant dose of the studied second-generation thiazolidinedione, rosiglitazone, given for a period of only 1 week decreases endogenous androgen production in healthy men. Obviously, the interpretation of our results is limited by this brief treatment period and the fact that we have investigated healthy men rather than diabetics. Further studies are needed to evaluate the importance of this observation in male diabetics who receive this agent on a long-term basis.

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