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SHORT COMMUNICATION

THE EFFECT OF AMINOGLUTETHIMIDE ON THE METABOLISM OF TESTOSTERONE IN RAT LIVER *IN VITRO*

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SUMMARY

Testosterone was incubated with liver slices of rats treated with aminoglutethimide (Elipten Ciba) for 7 or 14 days. Testosterone was metabolized more rapidly in the slices of both male and female liver after aminoglutethimide administration. Quantitative analysis of the testosterone metabolites indicated stimulation of ring A reduction and decrease of oxidation of the 17β-hydroxy group after aminoglutethimide treatment.

INTRODUCTION

SEVERAL years ago, aminoglutethimide (Elipten® Ciba, α-(*p*-aminophenyl)-α-ethylglutarimide), a drug originally used as an anti-convulsant, was found to inhibit adrenal steroidogenesis *in vivo* and *in vitro* [1–3]. The drug was shown to block steroidogenesis between cholesterol and 3β-hydroxy-5-pregnen-20-one, probably at the 20α-hydroxylation step [1, 2]; this block appears to be overcome in animals with normally functioning adrenopituitary feedback by increased ACTH secretion.

Beside influencing the biosynthesis of steroid hormones in the adrenals, aminoglutethimide affects the peripheral metabolism of steroids. Thus, catabolism of cortisol is influenced by inhibition of 11β-hydroxysteroid dehydrogenase and possibly also by stimulation of reduction of the 4-ene-3-keto group [4, 5]. The ratio of androsterone: etiocholanolone and of testosterone: epitestosterone was altered in a patient with ectopic ACTH-syndrome treated with aminoglutethimide [6]; simultaneous administration of testosterone and aminoglutethimide to hypogonadal males [7, 8] resulted in decreased excretion of testosterone, etiocholanolone, androsterone, and dehydroepiandrosterone as compared to the administration of testosterone alone. Clinically, unexplained light virilization occurs in some patients treated with aminoglutethimide.

Since these observations indicate that the metabolism of not only endogenous but also exogenous androgens is influenced by the drug, it seemed justifiable to investigate the effect of aminoglutethimide administration on testosterone degradation in liver tissue in more detail.

EXPERIMENTAL

Female and male Wistar rats of 250 g average weight, kept on Larsen's diet and offered tap water *ad lib.*, were injected subcutaneously with an aqueous

Table 1. The effect of aminoglutethimide administration on *in vitro* metabolism of [1,2-^{3}H] testosterone in liver slices of male and female rats. Each value represents an average of 6 determinations ± S.D. The yields of metabolites are related in % to the total radioactivity recovered after chromatography

Testosterone metabolite	Controls: 1 week		Aminoglutethimide: 1 week		Controls: 2 weeks		Aminoglutethimide: 2 weeks	
	Males yield %	Females yield %	Males yields %	Females yields %	Males yields %	Females yields %	Males yields %	Females yields %
Polar metabolites[a]	44·9 ± 7·1	35·7 ± 6·9	49·2 ± 8·1	52·3 ± 9·2*	46·7 ± 10·1	39·3 ± 6·8	52·4 ± 10·1	36·7 ± 7·7
5α-androstane-3α,17β-diol	13·0 ± 2·8	13·2 ± 3·1	16·5 ± 3·1	14·2 ± 2·1	12·1 ± 2·3	12·9 ± 2·1	17·8 ± 2·9*	21·1 ± 3·4*
Other androstanediols	12·9 ± 2·3	8·6 ± 1·8	15·3 ± 1·9	8·4 ± 1·3	11·7 ± 1·7	9·7 ± 2·2	16·2 ± 3·1*	13·0 ± 3·9
Testosterone	10·5 ± 2·4	13·0 ± 2·3	3·2 ± 1·0*	5·3 ± 1·2*	10·4 ± 3·1	11·2 ± 1·9	4·2 ± 0·8*	7·2 + 1·2*
17β-hydroxy-5α-androstan-3-one + 17β-hydroxy-5β-androstan-3-one[b]	2·4 ± 0·8	11·5 ± 2·4	3·5 ± 1·1	11·7 ± 2·3	2·7 ± 0·9	11·2 ± 2·7	4·1 ± 1·1	14·6 ± 3·3
3α-hydroxy-5α-androstan-17-one	13·1 ± 2·4	13·4 ± 2·2	9·1 ± 1·6*	6·7 ± 1·0*	12·9 ± 2·8	11·1 ± 2·6	3·9 ± 1·4*	4·9 ± 1·8*
3α-hydroxy-5β-androstan-17-one	2·4 ± 0·8	not found	1·2 ± 0·3	not found	2·3 ± 0·8	not found	1·1 ± 0·5	Not found
5α-androstane-3,17-dione + 4-androstene-3,17-dione	1·0 ± 0·2	4·6 ± 1·2	0·5 ± 0·1*	1·5 ± 0·3*	1·2 ± 0·2	4·7 ± 0·9	0·3 ± 0·1*	2·5 ± 0·4*

[a]Partially monohydroxy derivatives of testosterone: 7α-hydroxy-, 2β-hydroxy-, 6β-hydroxy- and 16α-hydroxy-testosterone were characterised chromatographically.

[b]17β-hydroxy-5β-androstan-3-one was not formed in female liver slices.

*Significance of difference from the appropriate control at the level $p < 0{\cdot}02$.

solution of aminoglutethimide phosphate (kindly supplied by CIBA, Basel) in daily doses of 20 mg per animal for one or two weeks. Untreated rats served as controls. Each experimental group consisted of 6 animals.

The rats were sacrificed by decapitation following a head blow, and liver slices (300 ± 20 mg) were incubated without added cofactors in 3 ml of Krebs–Ringer phosphate buffer (with 20 mmol glucose/liter), pH 7·4, with 0·5 μCi [1,2-^{3}H] testosterone (specific activity 41·8 Ci/mmol) at 37° for 60 min in an oxygen atmosphere.

The incubation mixtures were extracted twice with 10 ml of dichloromethane and the organic phase was washed with water and evaporated to dryness *in vacuo*. The dry residue was chromatographed on Whatman No. 1 paper in the system chloroform : *n*-hexane (1 : 1)/ethylene glycol. The individual zones were re-chromatographed and identified as described in detail elsewhere [9].

Radioactivity was scanned in a Packard-Radiochromatogram Scanner, Model 7201, and additionally measured in a Tri-Carb scintillation spectrometer. For details see reference [9].

RESULTS AND DISCUSSION

The yields of individual C_{19}-steroids formed from testosterone in liver slices from aminoglutethimide-treated rats and from controls are shown in Table 1.

The data on metabolite formation demonstrate a more rapid disappearance of testosterone from the incubation mixture in the experiments with liver slices from both male and female aminoglutethimide-treated rats. This, and a lower yield of 4-androstene-3,17-dione, are in accord with the observations of aminoglutethimide stimulation of cortisol ring A reduction [4, 5]. Some enhancement of testosterone hydroxylation by aminoglutethimide can be seen; however, it is significant only in females treated for one week. Significantly lower yields of 17-ketones and higher yields of 5α-androstane-3α,17β-diol and other androstanediols following prolonged aminoglutethimide administration prove 17β-hydroxysteroid dehydrogenase activity to have been depressed.

These facts may explain the effect of aminoglutethimide on the fate of exogenous testosterone in hypogonadal men [7, 8]: the excretion of the main 17-ketosteroids was diminished although testosterone was metabolized at a higher rate. The difference may depend partly on higher androstanediol and hydroxy-derivative formation. Owing to the sexual dimorphism [10] of steroid 5α- and 5β-dehydrogenation in the rat liver, only the male liver can be used as a model for 5α- and 5β-androstane derivative formation in man. However, changes in the androsterone : etiocholanolone ratio, observed in the ectopic ACTH-syndrome following aminoglutethimide treatment, were not significant in the present experiments with liver slices of aminoglutethimide treated male rats.

The changes in hepatic testosterone metabolism induced by aminoglutethimide were not accompanied by alterations of the optical-microscopic appearance of the liver tissue.

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