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Therapy of Cushing's Syndrome with Steroid Biosynthesis Inhibitors

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Several substances with different inhibitory effects on adrenal steroid biosynthesis were investigated in patients with Cushing's syndrome. It has been shown that trilostane, a 3β-hydroxysteroid-dehydrogenase inhibitor, is not potent enough to block cortisol biosynthesis in patients with hypercortisolism. Aminoglutethimide inhibits side chain cleavage of cortisol synthesis, but it has been demonstrated that the blocking effect on cortisol secretion is not strong enough to normalize urinary cortisol excretion in patients with Cushing's disease. For metyrapone, an inhibitor of adrenal 11β-hydroxylase, promising results were reported for the treatment of Cushing's syndrome. However, the drug has several side effects and depending on the definition of the desired reduction of cortisol secretion a true remission was only found in a minority of patients. The antifungal drug ketoconazole *in vitro* predominantly blocks 17,20-desmolase (IC_{50} 1 μM) and to a lesser extent 17α-hydroxylase (IC_{50} 10 μM) and 11β-hydroxylase (IC_{50} 15–40 μM). Therefore, ketoconazole *in vivo* most potently suppresses androgen secretion and only to a lesser extent cortisol biosynthesis. Several therapeutic trials with ketoconazole treatment in patients with pituitary Cushing's disease showed various remission rates between 30 and 90%. In contrast, in almost all patients with benign, primary adrenal Cushing's syndrome cortisol levels were normalized. In patients with ectopic ACTH syndrome ketoconazole was effective in about 50% of all reported cases, while cortisol hypersecretion due to adrenocortical carcinoma was only rarely inhibited by ketoconazole. The main side effect of ketoconazole treatment was liver toxicity which occurred in 12% of all treated patients. In contrast to ketoconazole, the narcotic drug etomidate shows a strong inhibitory effect on 11β-hydroxylase (IC_{50} 0.03–0.15 μM) but only a weak inhibition of 17,20 desmolase (IC_{50} 380 μM). This correlates with *in vivo* studies where even low, non-hypnotic doses of etomidate induced a pronounced fall in serum cortisol levels in normals and in patients with Cushing's syndrome. However, its clinical use is limited by its mandatory intravenous application and its sedative effects. In conclusion, ketoconazole remains the only available steroid-inhibitory drug for a therapeutic trial in patients with Cushing's syndrome who cannot be treated definitively by surgery.

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INTRODUCTION

Definitive surgical treatment is the therapy of choice in all forms of Cushing's syndrome; however, the outcome of surgical treatment largley depends on the etiology of the cortisol excess. A pituitary hypersecretion of ACTH (Cushing's disease) is found in 80% of all Cushing's syndromes and can be cured by transphenoidal pituitary microsurgery in about 80% of all patients. A primary adrenal cause of the hypercortisolism (unilateral adenoma, primary bilateral hyperplasia, carcinoma) contributes to 10% of all Cushing's syndromes. Except for rare cases with adrenocortical carcinoma most of these patients can be cured by a uni- or bilateral adrenalectomy. In about 10% of all patients, an ectopic, non-pituitary secretion of ACTH by a benign or malign tumor can be found but often the tumor is inoperable or cannot be located. Radiation therapy of the pituitary shows an effect only after several months or years and is effective only in a group of patients with persistent Cushing's disease. Therefore, about one-third of all patients with Cushing's syndrome require temporary or permanent medical

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Abbreviations: ACTH, adrenocorticotropic hormone; IC_{50}, drug concentration producing 50% inhibition *in vitro*.

treatment [1]. Out of the different available classes of potential therapeutic agents (steroidogenic blocking drugs, adrenolytic drugs, neuromodulatory drugs and steroid receptor antagonists) steroid biosynthesis inhibitors have the greatest importance. This paper will give an overview about the role of steroidogenic blocking agents in the treatment of Cushing's syndrome, based on published data in the literature as well as on our own *in vitro* and *in vivo* studies.

TRILOSTANE

Trilostane, an androstane-carbonitrile derivative, selectively blocks *in vitro* the 3β-hydroxysteroid-Δ5,4-isomerase system, which converts pregnenolone to progesterone [2]. Unfortunately, the clinical results in patients with Cushing's syndrome are disappointing. Only 3 of 13 treated patients showed a biochemical response, so this drug cannot be recommended [3, 4].

AMINOGLUTETHIMIDE

Aminoglutethimide, an anticonvulsant drug, inhibits *in vitro* the side chain cleavage of cholesterol to pregnenolone [5]. However, treatment of 39 patients with Cushing's disease only in 18 patients resulted in a complete remission [6]. Similarly, when combined with metyrapone treatment a normalization of urinary cortisol excretion was only found in 3 out of 6 patients with different forms of Cushing's syndrome [7].

METYRAPONE

The pyridine derivative metyrapone at low concentrations inhibits the transformation of 11-deoxycorticosterone to corticosterone (11β-hydroxylase) and at higher concentrations the side chain cleavage of cholesterol [8, 9]. In a recent study 91 patients with different forms of Cushing's syndrome were treated with metyrapone [10]. The authors reported a very good response to metyrapone treatment with remissions in 40/53 patients with Cushing's disease, 12/15 patients with adrenocortical tumors and in 17/17 patients with ectopic ACTH syndrome. However, they defined the therapeutic target range of mean serum cortisol levels/24 h to be <400 nmol/l (14.3 μg/dl). This is in contrast to the criteria of Orth and Liddle [11, 12] who define curative treatment from Cushing's syndrome with mean cortisol levels of <280 nmol/l (10 μg/dl). When this therapeutic target range is applied to the data of Verhelst *et al.* [10] metyrapone treatment is effective only in 10/53 patients with Cushing's disease, 7/15 patients with adrenocortical tumors and 4/17 patients with ectopic ACTH syndrome. Several side effects were observed during metyrapone treatment, especially hirsutism (16/43), dizziness (12/91) as well as edema, hypokalemia, nausea and rash [10]. Clinical data from other groups about the efficacy of metyrapone in patients with Cushing's syndrome are difficult to interpret because of concomitant treatment with aminoglutethimide or valproate [7,13].

KETOCONAZOLE

In 1983 we reported [14] that ketoconazole treatment (600 mg/day) in a patient with a cortisol producing adrenocortical adenoma induced a sharp fall in serum cortisol levels below 2.5 μg/dl. Incubation studies with tissue slices from this tumor and rising concentrations of ketoconazole resulted in a dose-dependent decrease of cortisol production *in vitro*. Further extensive incubation studies with human adrenocortical tissue slices and labeled precursors showed an inhibitory effect of the antimycotic imidazole derivative ketoconazole on the following adrenal enzymes: most potently ketoconazole blocked the C17,20-desmolase (conversion of 17α,20α-dihydroxyprogesterone to androstenedione) with an IC_{50} of 1 μM and to a lesser extent 17α-hydroxylase (conversion of progesterone to 17α-hydroxyprogesterone) with an IC_{50} of 10 μM and the 11β-hydroxylase (conversion of 11-deoxycortisol to cortisol; IC_{50} was 15–40 μM), 16α- and 18-hydroxylase. No inhibition was found for the 21-hydroxylase and 3β-hydroxysteroid-Δ5,4-isomerase activity [15, 16]. In analogy to these *in vitro* data the administration of ketoconazole (600 mg/day) in patients with normal adrenocortical function induced a pronounced rise of serum levels of 17α-hydroxyprogesterone, 11-deoxycortisol, 11-deoxycorticosterone and corticosterone, but only a slight fall of serum cortisol (Fig. 1) [17]. In 15 patients with hyperandrogenism treatment with ketoconazole induced a fall in serum levels of dehydroepiandrosterone-sulfate (DHEA-S), dehydroepiandrosterone (DHEA), androstenedione and testosterone by 30–50% while cortisol levels were reduced by only 19% (Fig. 2) [18]. Thus *in vitro* and *in vivo* the blocking effect of ketoconazole on androgen

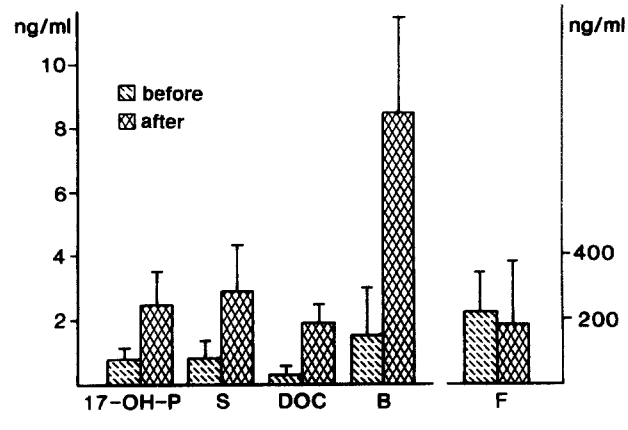

Fig. 1. Serum levels of 17α-hydroxyprogesterone (17-OH-P), 11-deoxycortisol (S), 11-deoxycorticosterone (DOC), corticosterone (B) and cortisol (F) before and after 600 mg ketoconazole in 3 patients with normal adrenocortical function [17].

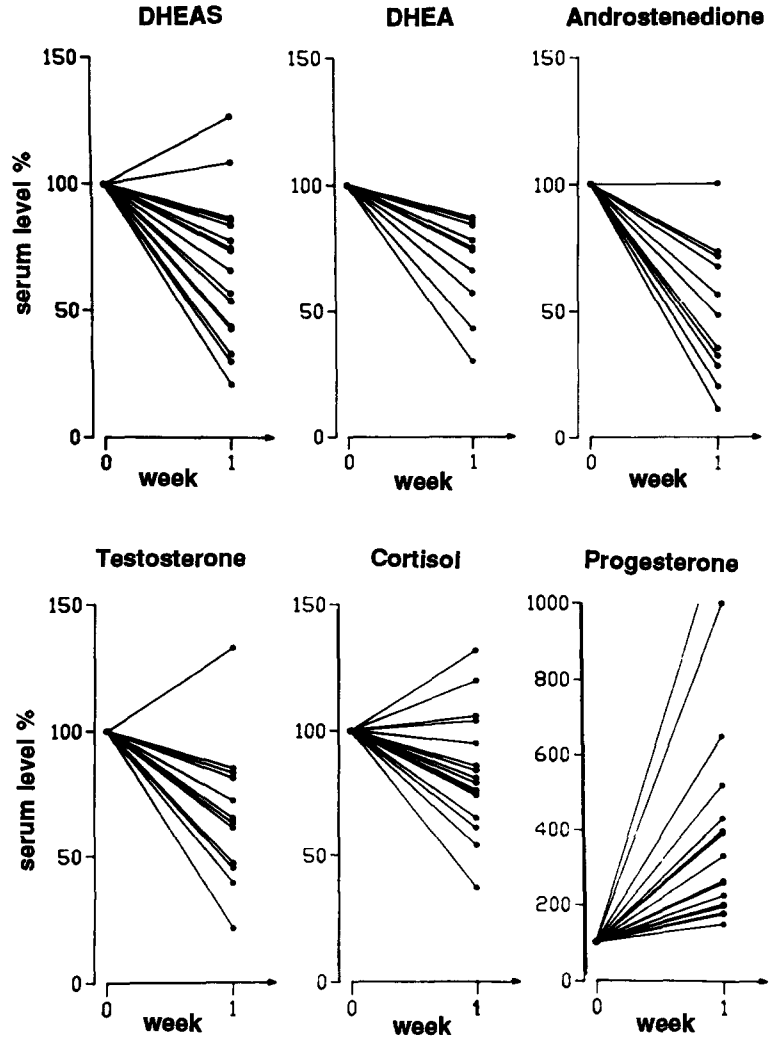

Fig. 2. Serum levels of DHEA-S, DHEA, androstenedione, testosterone, cortisol and progesterone before and after 600 mg ketoconazole in 15 patients with hyperandrogenism of various origin, expressed in percent of control [18].

biosynthesis is much more pronounced than the inhibition of cortisol biosynthesis.

For the treatment of Cushing's syndrome several therapeutic trials with ketoconazole have been published [19–30]. Table 1 summarizes the results of ketoconazole treatment in 82 patients with Cushing's disease. The rate of remissions due to the treatment, based on normalization of free cortisol in the 24-h urine, varied between 25 and 93%. The average remission rate was 70% and the response to ketoconazole did not seem to be time- or dose-dependent. The main side effect was liver toxicity which occurred in about 12%

Table 1. Summary of published results of ketoconazole treatment in 82 patients with Cushing's disease [19–26]

Authors	*n*	Dose mg	Duration w, m, y	Normalization urine cortisol	Side effects
Loli *et al.*, 86	7	600–800	3 m	3/7	0/8
McCance *et al.*, 87	6	800	– 1 w	5/6	3/6 Liver toxicity
Diop *et al.*, 89	5	800–1200	8 m	1/5	0/5
Cerdas *et al.*, 89	7	600	1 w	7/7	1/7 Liver toxicity 3/7 Oedema
Tabarin *et al.*, 91	4	400–1200	4 w–6 m	1/4, 4/4	0/4
Mortimer *et al.*, 91	8	800	2 w	8/8	2/8 Liver toxicity
Sonino *et al.*, 91	28	400–800	3 w–3 y	26/28	4/34 Liver toxicity
Engelhardt *et al.*, 89, 93	17	600	1 w–1 y	6/17	3/29 Liver toxicity
				57/82 (70%)	16/108 (15%)

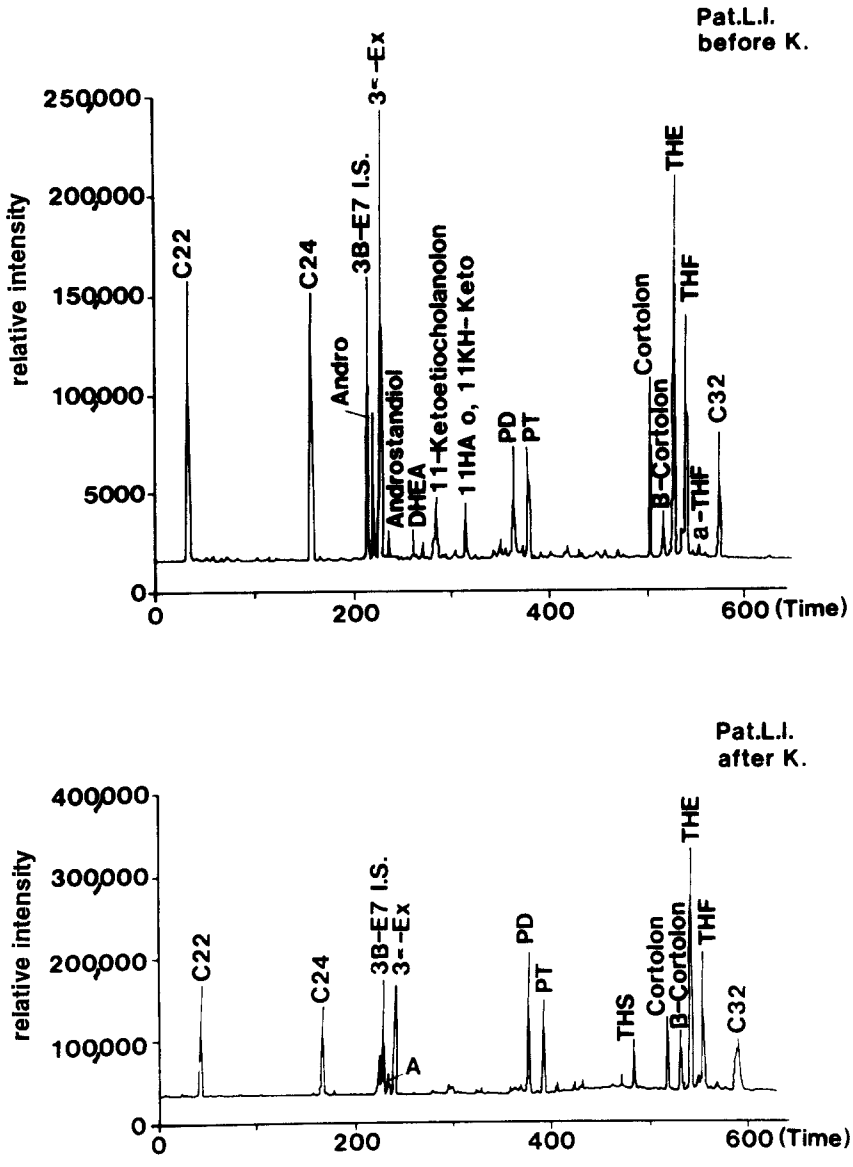

Fig. 3. Gas chromatographic separation of 24-h urine from a patient with Cushing's disease and no response to ketoconazole before and after 600 mg ketoconazole.

of all reported cases. In our own study with 17 patients with pituitary Cushing's disease we found a rather disappointing remission rate of only 6/17. The insufficient suppression of cortisol biosynthesis by ketoconazole in a number of patients with Cushing's disease might be due to an increase in ACTH secretion which could overcome the steroidogenic block. This hypothesis is supported by the observation that mean ACTH serum levels in 6 patients with remission during ketoconazole treatment only rose by 35%, whereas in 11 patients without normalization of urinary cortisol a mean increase of ACTH by 80% was found during ketoconazole therapy. A sensitive indicator for normalization of cortisol excretion is the quotient of tetrahydro-11-deoxycortisol (THS) to tetrahydrocortisol (THF) found by gas chromatographic analysis of a 24-h urine sample. As shown in Figs 3 and 4, this THS/THF quotient was below 0.5 in a patient without response and about 10 in a patient with remission due to ketoconazole treatment.

The results of ketoconazole treatment in other forms of Cushing's syndrome are summarized in Table 2. All 7 patients with an adrenal adenoma [19, 24, 26] and all 5 patients with primary bilateral hyperplasia [24, 26, 28] showed a normalization of urinary cortisol excretion during ketoconazole treatment. Furthermore, 4 out of 9 patients with an ectopic ACTH syndrome showed a remission of the cortisol excess [24–26, 29], but only 1 out of 5 patients with adrenocortical carcinoma [24, 26, 27, 30].

ETOMIDATE

The imidazole derivative etomidate is a short-acting hypnotic substance which is only effective when given intravenously. Incubation studies with human adrenocortical tissue showed that it predominantly blocks the conversion of 11-deoxycortisol to cortisol (IC_{50} 0.15 μM) and of 11-deoxycorticosterone to corticosterone (IC_{50} 0.03 μM) by the 11β-hydroxylase (Fig. 5) [16]. Thus etomidate is 10- to 100-fold more potent in inhibiting adrenal 11β-hydroxylase activity than ketoconazole and is the most potent inhibitor of the adrenal enzyme system we know. The blocking effect of etomidate and ketoconazole on adrenal 17α-hydroxylase activity is comparable with IC_{50}s of approx. 10 μM [16]. However, the blocking effect on the conversion of 17α-hydroxyprogesterone to androstenedione (C17,20-desmolase activity) is much lower for etomidate (IC_{50} 380 μM) than for ketoconazole (IC_{50} 1 μM, Fig. 6) [16].

Corresponding to these *in vitro* data, etomidate effectively suppressed cortisol secretion in patients with normal adrenocortical function or with hypercortisolism. In a prospective, comparative study the effect of intravenous thiopental or etomidate on serum cortisol and ACTH levels was tested in 9 healthy men [31]. The mean serum cortisol levels were significantly lower in individuals receiving etomidate (5.0 μg/dl) in comparison to thiopental (90 μg/dl). Mean serum ACTH levels remained unchanged after thiopental but rose by 100% after the injection of etomidate [31]. In 5 patients with Cushing's syndrome the infusion of non-sedating low doses of etomidate for 3 days induced a rapid fall of mean cortisol levels (from 40 to 20 μg/dl) already after 7 h to a steady-state level of about 12 μg/dl after

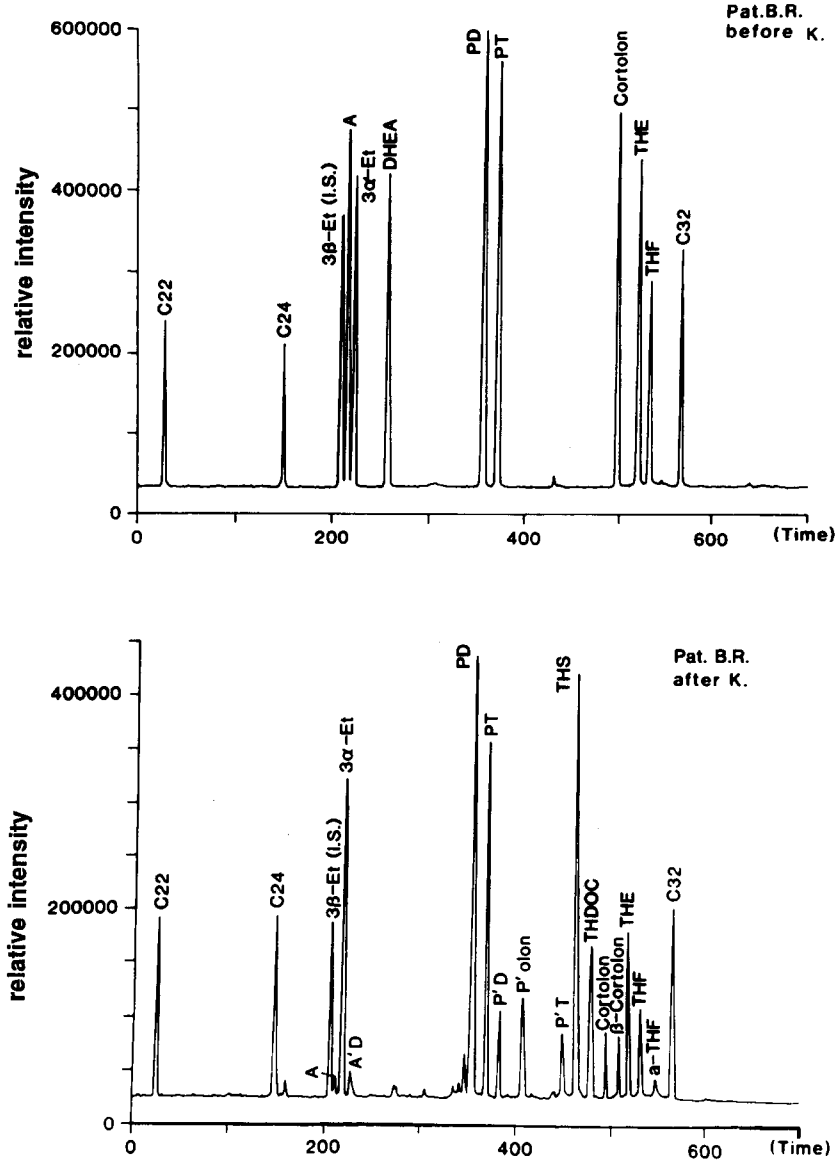

Fig. 4. Gas chromatographic separation of 24-h urine from a patient with Cushing's disease and response to ketoconazole before and after 600 mg ketoconazole.

Table 2. Summary of published results of ketoconazole treatment in 26 patients with different forms of Cushing's syndrome [19, 24–30]

	n	Duration w, m	Normalization urinary cortisol	
Adrenal adenoma				
Loli *et al.*, 86	1	2 m	1/1	7/7
Sonino *et al.*, 91	1	3 w	1/1	
Engelhardt *et al.*, 89, 93	5	1–3 w	5/5	
Prim. adrenal hyperplasia				
Oelkers *et al.*, 86	1	4 w	1/1	5/5
Sonino *et al.*, 91	2	4 w, 5 m	2/2	
Engelhardt *et al.*, 89, 93	2	1 w, 1 m	2/2	
Adrenal carcinoma				
Sonino *et al.*, 91	1	6 m	0/1	1/5
Engelhardt *et al.*, 89, 93	3	1–6 m	0/3	
Sinnaeve *et al.*, 89	1	2 w	1/1	
Ectopic ACTH syndrome				
Sonino *et al.*, 91	2	2, 14 m	1/2	4/9
Tabarin *et al.*, 91	4	1–3 m	0/4	
Farwell *et al.*, 91	1	3 m	1/1	
Engelhardt *et al.*, 89	2	3, 6 m	2/2	

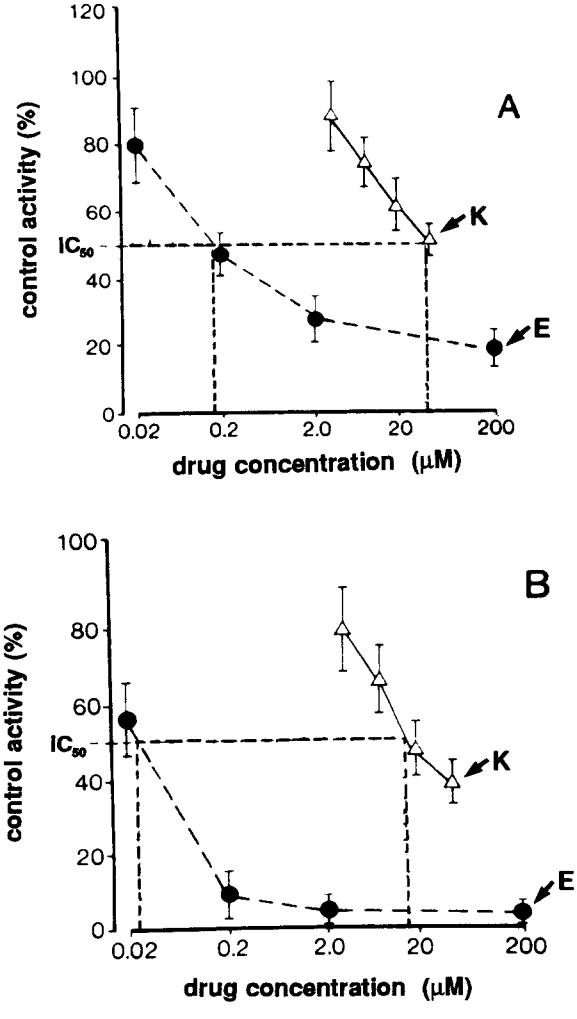

Fig. 5. Inhibition of the conversion of tritiated 11-deoxycortisol to cortisol (A) and 11-deoxycorticosterone to corticosterone (B) in human adrenocortical tissue slices by rising concentrations of ketoconazole (K) and etomidate (E) [17].

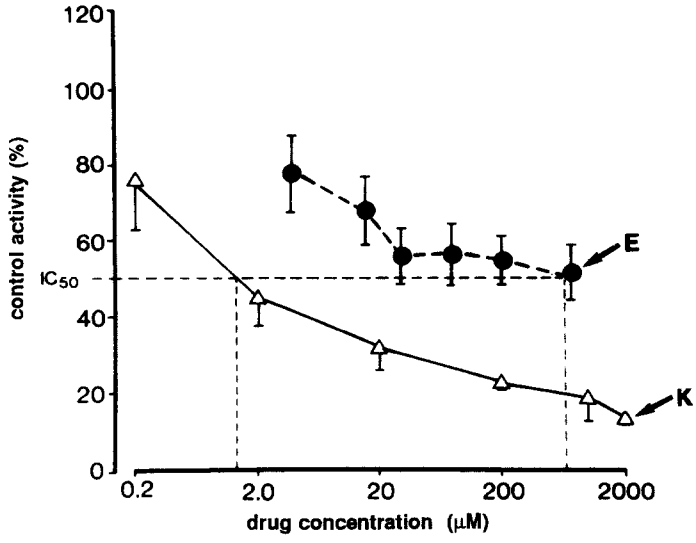

Fig. 6. Inhibition of the conversion of tritiated 17α-hydroxyprogesterone to androstenedione in human adrenocortical tissue slices by rising concentrations of ketoconazole (K) and etomidate (E) [17].

3 days [32]. These data show that in analogy to the *in vitro* results etomidate in comparison to ketoconazole has a stronger inhibitory potency on cortisol biosynthesis in patients with Cushing's syndrome. Unfortunately, etomidate has to be given intravenously and can therefore not be used for continuous therapy.

CONCLUSIONS

On the basis of the published data on the therapeutic use of the available steroid biosynthesis inhibitors—metyrapone, ketoconazole and etomidate—in patients with different forms of Cushing's syndrome, we conclude that ketoconazole seems to be the drug of choice for medical treatment of Cushing's disease. A therapeutical trial with ketoconazole should be attempted in patients who have non-curable forms of Cushing's syndrome. For remission, a normalization of urinary excretion of free cortisol is desired and liver enzymes should be controlled within short intervals during therapy.

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