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INHIBITION OF STEROIDOGENIC CYTOCHROME P-450 ENZYMES IN RAT TESTIS BY KETOCONAZOLE AND RELATED IMIDAZOLE ANTI-FUNGAL DRUGS

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Summary—Ketoconazole, an imidazole antifungal drug, has previously been shown to diminish testosterone and cortisol production in patients as well as rat and mouse cells *in vitro*. Inhibition of adrenal mitochondrial cytochrome P-450 enzymes was demonstrated. In this study we tested several imidazole antifungal drugs and examined the individual steps in testicular steroidogenesis to determine which enzymes in the androgen pathway were blocked. In addition, we studied 25-hydroxyvitamin D 24-hydroxylase activity in cultured pig kidney cells (LLC-PK_1) to assess a mitochondrial P-450 enzyme in another organ. All imidazoles tested inhibited both total testosterone production and 24-hydroxylase activity but the relative potencies differed. We next studied the individual testicular enzymatic steps between cholesterol and testosterone. Ketoconazole inhibited cholesterol-side-chain-cleavage enzyme (mitochondrial) and C-17,20 lyase (microsomal). The three inhibited enzymes (two testicular and one renal) are all P-450 cytochromes. Testicular 17-hydroxylase, also a P-450 cytochrome, was not inhibited even at high doses of ketoconazole. This is an interesting finding because the testicular hydroxylase and lyase have been shown to be a single protein. Non-cytochrome P-450 enzymes in the androgen pathway were not inhibited. The results demonstrate that several imidazole antifungal drugs all inhibit both microsomal and mitochondrial cytochrome P-450 enzymes in multiple organs.

INTRODUCTION

Imidazole antifungal drugs have become a major therapeutic modality in the treatment of mycotic infections. Because ketoconazole (Nizoral, Janssen Pharmaceutica, Beerse, Belgium) can be administered orally and has a broad spectrum of activity against both superficial and deep mycoses, this imidazole derivative is a widely used antifungal agent worldwide [1]. Although well-tolerated, ketoconazole has recently been noted to inhibit both adrenal and testicular steroidogenesis [2–7]. Inhibition of testosterone production was shown both *in vivo* in patients [3, 6, 7] and *in vitro* after gonadotropin stimulation of isolated rodent Leydig cells [3, 7]. Ketoconazole has also been shown to inhbit ACTH-stimulated cortisol production both in volunteers and rat adrenal cells *in vitro* [2].

It has been known for some time that imidazole derivatives inhibit microsomal cytochrome P-450 mixed function oxidases, probably by interacting with the heme iron of the cytochrome [8, 9]. The antifungal activity of the imidazole drugs is thought to be related to their ability to inhibit fungal cytochrome P-450 enzymes, especially the C-14 demethylation of lanosterol in the pathway to ergosterol [10, 11]. In recent studies [5] into the mechanism by which ketoconazole inhibits mammalian adrenal steroidogenesis, we have demonstrated that the drug decreased the activity of two rat adrenal mitochondrial cytochrome P-450 enzymes, the cholesterol-side-chain cleavage enzyme and 11-β hydroxylase; non-mitochondrial cytochrome P-450 enzymes were not blocked. In the present paper we have extended our studies in two directions. First, we examined several imidazole antifungal drugs to determine whether inhibition of cytochrome P-450 enzymes in steroidogenic pathways is a general property of this group of drugs or whether this was a unique property of ketoconazole. The drugs tested include, in addition to ketoconazole, clotrimazole, *trans*-ketoconazole and RS49910. Clotrimazole is a potent antifungal agent used primarily as a topical agent [12]. *Trans*-ketoconazole (the *trans*-isomer of ketoconazole) has reduced anti-fungal activity compared to ketoconazole and differs in the spatial orientation of the piperazine side chain. RS49910 is a substituted aryl alkylimidazole experimental antifungal drug from Syntex. The second new direction in this paper is our attempt to ascertain which specific enzymes are inhibited in the androgen pathway. Indirect evidence suggests that C-17,20 lyase, a microsomal cytochrome P-450 enzyme, may be an important step in the androgen blockade by ketoconazole [6]. If so, this would be substantially different than the glucocorticoid pathway where only mitochondrial cytochrome P-450 enzymes appeared to be blocked in the adrenal gland [5].

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EXPERIMENTAL

Materials

[^{3}H]Testosterone (60 Ci/mmol), [^{3}H]25-hydroxyvitamin D_3 (22 Ci/mmol), [^{3}H]cholesterol (5 Ci/mmol), [^{3}H]pregnenolone (21 Ci/mmol), [^{3}H]progesterone (50 Ci/mmol), [^{3}H]androstenedione (85 Ci/mmol) and [^{3}H]17-hydroxyprogesterone (50 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). HCG was obtained from the National Institute of Child Health and Development, NIH (Bethesda, MD). Testosterone was purchased from Steraloids (Wilton, N.H.). Ketoconazole (Fig. 1) was obtained from Janssen Pharmaceutica (Beerse, Belgium) and clotrimazole (Fig. 1) from Schering (Kenilworth, NJ). RS49910 and *trans*-ketoconazole were kindly provided by Syntex (Palo Alto, CA). Culture media were obtained from Grand Island Biological (Grand Island, N.Y.).

Testosterone production by intact Leydig cells

Interstitial cell preparations containing Leydig cells were prepared from the testes of 200 g Sprague–Dawley rats as described by Azhar and Menon[13]. This interstitial cell preparation has been found to contain approx 15% Leydig cells [14]. In brief, the testes were decapsulated and incubated at 37°C for 20 min with 0.25 mg/ml collagenase (Worthington, Type II, Freehold, NJ) in Medium 199 containing 2 mg/ml fatty acid poor bovine serum albumin (BSA) [Calbiochem, La Jolla, CA]. The supernatant, containing Leydig cells, was decanted through nylon mesh to remove debris. The cells were washed by centrifugation and then utilized to study testosterone production. Cells were incubated at 37°C under 5% CO_2–95% O_2 in plastic scintillation vials containing Medium 199 with 0.1% BSA and 2.5 mM $CaCl_2$ in a total volume of 1 ml. Following a 15 min equilibration period, cells were treated with vehicle or drug for 30 min prior to addition of 100 ng/ml hCG. After a 2 h treatment period the incubation was terminated by centrifugation, and the supernatant collected and stored at −20°C for future analysis. Testosterone was measured by radioimmunoassay employing antibody No. 250-11-BSA from Niswender *et al.*[15].

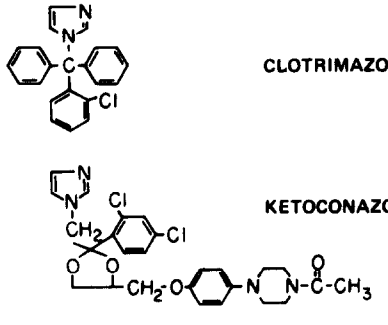

Fig. 1. Structure of ketoconazole and clotrimazole.

Cholesterol-side-chain-cleavage enzyme

Testes were decapsulated and then disrupted with a Teflon-glass homogenizer in 0.25 M sucrose, 20 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 500 *g* to remove nuclei and cell debris. The mitochondrial fraction was obtained by subsequent centrifugation and washing at 9,000 *g* in a Sorvall RC-5 refrigerated centrifuge. The supernatant was used for the microsomal fraction experiments (see below). Cholesterol-side-chain-cleavage product formation was assessed by the conversion of [^{3}H]cholesterol to [^{3}H]pregnenolone and other products in the presence of NADPH and Krebs cycle intermediates as described by Van der Vusse *et al.*[16]. The reaction was stopped by the addition of methylene chloride (5 ml); steroids were completely extracted with two additional methylene chloride washes. The steroid products were assessed on a Varian model 5000 high performance liquid chromatograph (HPLC) equipped with a reverse phase Varian MCH-10 column (Varian Associates, Inc., Palo Alto, CA). The cell and media extracts were chromatographed in a methanol–water system with stepwise increments in the methanol concentration. Radioinert steroid standards (Steraloids Inc., Wilton, NH) were used for calibration.

Testicular microsomal enzymes

The supernatant resulting from the 9000 *g* centrifugation (described above) was subjected to subsequent centrifugation at 105,000 *g* for 1 h in a Beckman L5-75B ultracentrifuge to obtain the microsomal fraction. The pelleted microsomes were incubated in Krebs–Ringer bicarbonate buffer with [^{3}H]progesterone, [^{3}H]pregnenolone, [^{3}H]17-hydroxyprogesterone or [^{3}H]androstenedione in 1 ml volumes at 37°C for 30 min under 95% O_2–5% CO_2 as described by Nozu *et al.*[17]. The reaction was stopped by the addition of methylene chloride (5 ml) and extracted as described above. The steroid products were analyzed by HPLC as above.

Renal 24-hydroxylase enzyme

Cultured pig kidney cells (LLC-PK_1) were grown to confluence in minimal essential medium, supplemented with 10% newborn calf serum as previously described [18]. 25-Hydroxyvitamin D-24-hydroxylase (24-hydroxylase) activity was then induced by treatment of cells with 1,25$(OH)_2D_3$ (13 nM) for 6–8 h. Enzyme product formation was assessed in whole cells by measuring the conversion of [^{3}H]25$(OH)D_3$ to [^{3}H]24,25$(OH)_2D_3$ by HPLC as previously described [19].

RESULTS

Effects of imidazole antifungal drugs on testosterone production

To elucidate whether imidazole antifungal drugs, as a class, inhibited testosterone production, freshly

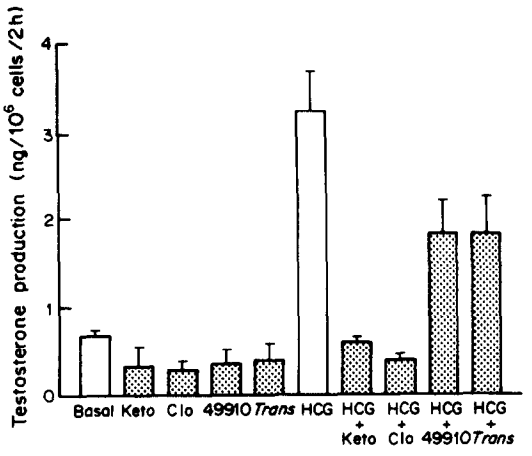

Fig. 2. Antifungal drug inhibition of basal and hCG stimulated Leydig cell testosterone production. Freshly isolated rat Leydig cells (7–10 × 10^6 cells/ml) were pre-incubated with a series of concentrations of antifungal drugs. Subsequently cells were challenged with hCG (100 ng/ml) or vehicle and incubated for an additional 2 h. Testosterone was measured by radioimmunoassay. Values shown are means ± SE, $n = 3$–5 duplicate assays for each point. For basal inhibition we have plotted the antifungal dose that showed testosterone inhibition: keto (ketoconazole 2 μM), clo (clotrimazole 2 μM), R49910 (RS49910 20 μM), *trans* (*trans*-ketoconazole 20 μM). For hCG stimulation, we have plotted the same concentration for all drugs (1 μM) so that comparative potency may be evaluated.

isolated Leydig cells were incubated in the presence of four imidazoles, and testosterone production was measured by RIA. Basal (unstimulated) testosterone production was inhibited by all the imidazole antifungal drugs tested (Fig. 2). HCG-stimulation caused a 5-fold rise in testosterone production. Antifungal treatment of the cells caused a marked inhibition of the hCG-stimulated testosterone production (Fig. 2). All four antifungal drugs inhibited testosterone production in a dose-dependent manner (Fig. 3). The relative potencies of the imidazoles, expressed as the dose required to inhibit 50% (ID_{50}) of the hCG-stimulated testosterone, were: clotrimazole = ketoconazole (0.2 μM) > RS49910 (0.86 μM) > *trans*-ketoconazole (1.1 μM). The ketoconazole inhibition was shown to be reversible by restoration of full responsiveness to hCG after the antifungal drug was washed out.

Effects of imidazole antifungal drugs on renal 24-hydroxylase activity

To establish whether the inhibitory actions were limited only to the testes, we next evaluated the same four imidazoles as inhibitors of the renal mitochondrial cytochrome P-450 enzyme, 24-hydroxylase. Figure 4 illustrates the dose-dependent inhibition of 24-hydroxylase. The relative potencies and ID_{50} values were: clotrimazole (0.2 μM) > ketoconazole (0.5 μM) > *trans*-ketoconazole (0.6 μM) > RS49910 (6 μM).

Effect of ketoconazole on cholesterol-side-chain-cleavage enzyme

We next attempted to identify the specific enzymatic steps in the testosterone pathway that were inhibited by ketoconazole. Cholesterol side chain

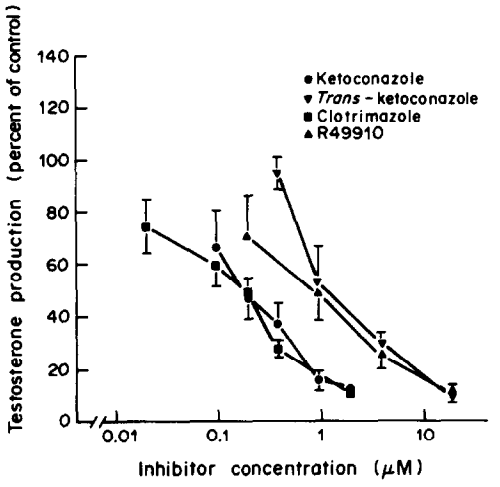

Fig. 3. Antifungal drug inhibition of hCG-stimulated Leydig cell testosterone production. Freshly isolated rat Leydig cells (7–10 × 10^6 cells/ml) were preincubated for 30 min with varying concentrations of antifungal drugs, then stimulated with hCG (100 ng/ml) and incubated for an additional 2 h. Testosterone in the medium was measured by radioimmunoassay. The results are expressed as a percent of the vehicle treated control (3.2 ± 0.45 ng testosterone/10^6 cells/2 h) which was taken as 100%. Values shown are means ± SE, $n = 3$–5 duplicate assays for each point.

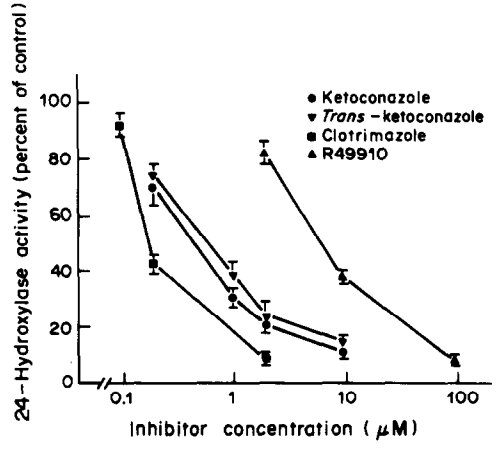

Fig. 4. Antifungal drug inhibition of $1,25(OH)_2D_3$ stimulated LLC-PK_1 cell 24-hydroxylase. Cultured pig kidney cells (LLC-PK_1) were grown to confluence in minimal essential medium. Cells were incubated with 13 nM $1,25(OH)_2D_3$ to induce 24-hydroxylase enzyme and harvested 6–8 h later. Various concentrations of antifungal drugs or vehicle were added and 24-hydroxylase activity was then assessed by measuring the conversion of [^{3}H]$25(OH)D_3$ substrate (0.5 μM) to [^{3}H]$24,25(OH)_2D_3$ using high performance liquid chromatography. Results are expressed as a percentage of the vehicle treated control cells (6.5 ± 0.63 pmol [^{3}H]$24,25(OH)_2D_3/10^6$ cells/30 min). Each value represents the mean ± SE, $n = 3$ duplicate assays for each point.

cleavage enzyme was studied by incubating [^{3}H]cholesterol substrate with a testicular mitochondrial fraction and conversion products were separated by HPLC (Fig. 5). In control cells, [^{3}H]cholesterol gave rise to [^{3}H]pregnenolone and small amounts were subsequently converted to [^{3}H]progesterone and [^{3}H]testosterone. Ketoconazole inhibited the cholesterol conversion to pregnenolone and more distal products in a dose-dependent fashion. Ketoconazole, at 50 μM, caused almost complete suppression of pregnenolone production. The ketoconazole concentration causing 50% inhibition was $\sim$0.3 μM.

Effect of ketoconazole on microsomal steroidogenic enzymes

We next examined the enzymatic conversion of [^{3}H]pregnenolone to [^{3}H]progesterone mediated by the non-cytochrome P-450 enzymes 5-ene-3β-hydroxysteroid dehydrogenase and steroid isomerase. A microsomal fraction was treated with ketoconazole or saline for 15 min, then incubated for an additional 30 min with [^{3}H]pregnenolone (49 nM) as substrate. In control cells, production of progesterone, and some subsequent conversion to other steroids was observed. Treatment with various doses of ketoconazole up to 50 μM failed to inhibit conversion to progesterone (data not shown). In fact, in the ketoconazole treated cells there was a dose-dependent accumulation of progesterone and 17-hydroxyprogesterone suggesting inhibition of a more distal enzyme.

Testicular microsomes were next incubated with [^{3}H]progesterone to examine the production of [^{3}H]testosterone. The microsomes freely converted this substrate to 17-hydroxyprogesterone and subsequently to androstenedione and testosterone (Fig. 6). Ketoconazole did not inhibit the cytochrome P-450 dependent enzyme, 17α-hydroxylase, so that conversion of progesterone to 17-hydroxyprogesterone was not impaired. In contrast, ketoconazole caused a dose-dependent inhibition of the cytochrome P-450 enzyme, C-17,20 lyase, as illustrated by a progressively increasing accumulation of 17-hydroxyprogesterone and decreasing production of testosterone (Fig. 6). The ketoconazole dose of 12 μM inhibited 50% of the C-17,20 lyase activity. No accumulation of androstenedione occurred in ketoconazole treated microsomes, indicating the non-cytochrome P-450 enzyme 17β-hydroxysteroid dehydrogenase was not inhibited, allowing almost complete conversion of any androstenedione produced to testosterone (Fig. 6).

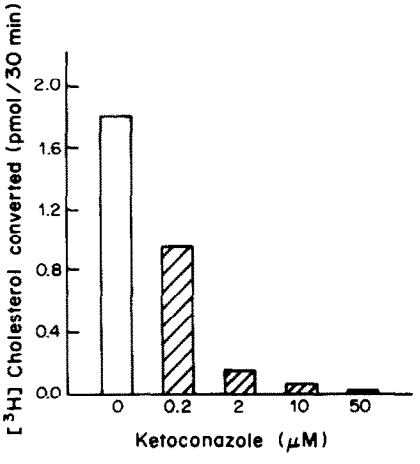

Fig. 5. Ketoconazole inhibition of cholesterol-side-chain-cleavage enzyme in a testicular mitochondrial preparation. A mitochondrial-enriched fraction was pretreated with varying doses of ketoconazole or saline vehicle for 15 min. [^{3}H]Cholesterol (230 nM) was added as a substrate and the incubation continued for 30 min. Extracts of cells and medium were chromatographed by reverse-phase HPLC in a methanol–water system. The initial methanol concentration was 60%, increasing stepwise to 80% at 15.5 min and then to 100% at 22 min. The standards were chromatographed in the same system and their elution times were pregnenolone (23.5 min), progesterone (21 min), 17-hydroxyprogesterone (16 min), androstenedione (14.5 min) and testosterone (18.5 min). The areas corresponding to all these peaks were summed and expressed as [^{3}H]cholesterol converted to pregnenolone plus other steroids.

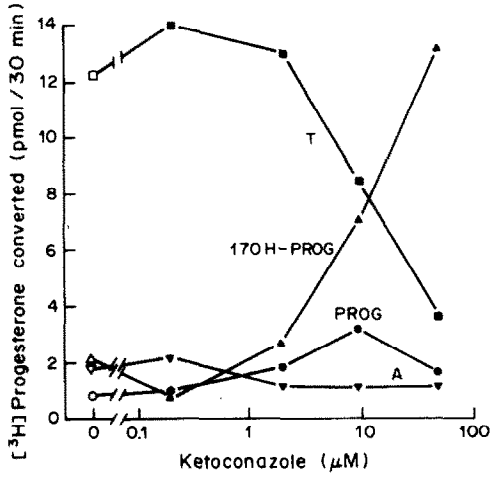

Fig. 6. Ketoconazole inhibition of testosterone production in a testicular microsomal preparation. A microsomal-enriched fraction was pre-treated with varying doses of ketoconazole (closed symbols) or saline vehicle (open symbols) for 15 min. [^{3}H]Progesterone (26 nM) was added as a substrate, and the incubation continued for 30 min. Extracts of cells and medium were chromatographed by reverse phase HPLC in a methanol–water system. The initial methanol concentration was 55%, increasing step-wise to 80% at 29 min. The standards were chromatographed in the same system and their elution times were progesterone (35 min), 17-hydroxyprogesterone (28 min), androstenedione (25 min) and testosterone (32 min). The area corresponding to each peak is expressed as [^{3}H]progesterone converted. The abbreviations are: progesterone (Prog), testosterone (T), androstenedione (A) and 17-hydroxyprogesterone (17-OH Prog).

Effect of ketoconazole on C-17,20 lyase

The effect of ketoconazole specifically on the C-17,20 lyase was examined by incubating microsomes with [^{3}H]17-hydroxyprogesterone as substrate (Fig. 7). In control samples, most substrate was converted to testosterone with some androstenedione detectable indicating the action of both C-17,20 lyase and 17β-hydroxysteroid dehydrogenase. As the ketoconazole concentration was increased, there was a dose dependent fall in testosterone ($ID_{50} \sim 10\ \mu M$) with no decrease in androstenedione production indicating specific inhibition of the C-17,20 lyase. To substantiate this finding, [^{3}H]androstenedione was incubated with microsomes (Fig. 8). Ketoconazole, up to 50 μM, failed to inhibit 17β-hydroxysteroid dehydrogenase. Thus any androstenedione produced using [^{3}H]progesterone (Fig. 6) or [^{3}H]17-hydroxyprogesterone (Fig. 7) was converted to testosterone.

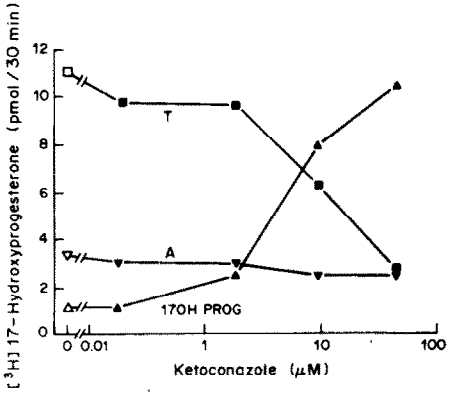

Fig. 7. Ketoconazole inhibition of testosterone production in a testicular microsomal preparation with [^{3}H]17-hydroxyprogesterone substrate. A microsomal-enriched fraction was pre-treated with varying doses of ketoconazole (closed symbols) or saline vehicle (open symbols) for 15 min. [^{3}H]17-hydroxyprogesterone (23 nM) was added as a substrate, and the incubation continued for 30 min. Extracts of cells and medium were chromatographed by reverse phase HPLC as described in Fig. 6. The area corresponding to each peak is expressed as [^{3}H]17-hydroxyprogesterone converted. The abbreviations are: 17-hydroxyprogesterone (17-OH-Prog), testosterone (T) and androstenedione (A).

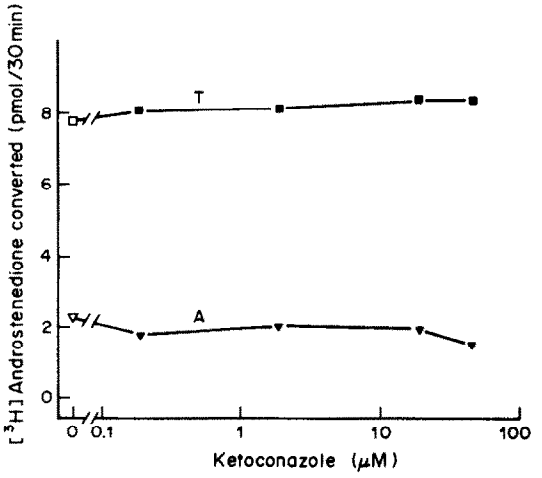

Fig. 8. Ketoconazole inhibition of testosterone production in a testicular microsomal preparation with [^{3}H]androstenedione substrate. A microsomal-enriched fraction was pre-treated with varying doses of ketoconazole (closed symbols) or saline vehicle (open symbols) for 15 min. [^{3}H]androstenedione (14 nM) was added as a substrate, and the incubation continued for 30 min. Extracts of cells and medium were chromatographed by reverse phase HPLC as described in Fig. 6. The area corresponding to each peak is expressed as [^{3}H]androstenedione converted. The abbreviations are: androstenedione (A) and testosterone (T).

DISCUSSION

The studies described in this paper demonstrate that the four imidazole antifungal drugs tested inhibited enzymatic pathways in two organs: testosterone production in rat Leydig cells and 24-hydroxylase activity in pig kidney cells. Examination of the specific enzymes blocked in the androgen pathway indicated that ketoconazole inhibited selective mitochondrial and microsomal cytochrome P-450 enzymes, but non-cytochrome P-450 enzymes were not inhibited. At least one microsomal cytochrome P-450 enzyme, 17α-hydroxylase, was also not inhibited.

A second finding of these experiments is that a variety of imidazole antifungal drugs act to inhibit multiple cytochrome P-450 enzyme systems in several mammalian organs, namely kidney, testes, and, in previous studies, adrenal glands [5]. This suggests that imidazole antifungal drugs may share the general property of inhibiting cytochrome P-450 enzymes in both fungi and multiple mammalian species [2–11]. Furthermore, the inhibitory potency of a particular imidazole antifungal drug can vary quite significantly depending on the tissue source. In this study (Table 1) the difference in ID_{50} of the antifungal drugs ranged from equipotent (ketoconazole) to more than a 6-fold difference (RS49910) when tested in pig kidney versus testis. Additionally, differences in inhibitory potencies may exist between species in view of a recent paper by Schurmeyer and Nieschlag [7] which showed murine testosterone production was inhibited by clotrimazole ($ID_{50} \sim 0.9\ \mu M$) and ketoconazole ($ID_{50} \sim 0.9\ \mu M$) compared to that of rat where the ID_{50} for both drugs was $\sim 0.2\ \mu M$ (Table 1). Thus, one might be able to utilize the hierarchy of potencies to design specific drugs to inhibit cytochrome P-450 enzyme systems in certain organs while possibly sparing those in another organ.

Table 1. Potency of antifungal drugs as inhibitors of cytochrome P-450 enzymes

	ID_{50} (μM)	
	Testosterone	24-Hydroxylase
Clotrimazole	0.2	0.2
Ketoconazole	0.2	0.5
RS49910	0.9	6.0
Trans-ketoconazole	1.1	0.6

Summary of data obtained from Figs 1–3.

Although testosterone inhibition by ketoconazole has been observed previously, both *in vitro* [3, 7] and *in vivo* [3, 6, 7], the precise enzymatic steps which were affected had not previously been identified. Because basal testosterone production was inhibited, it seemed likely that the locus of inhibition was not related to either hCG receptor binding or cAMP production. In analogous experiments in rat adrenal glands, we had previously shown that ketoconazole inhibited corticosterone synthesis at sites beyond receptor binding and cAMP generation [5]. Since cholesterol-side-chain-cleavage enzyme was inhibited by ketoconazole in rat adrenal cells [5], we suspected this enzyme would similarly be inhibited in rat testes. Our study demonstrates an inhibition of rat testicular cholesterol-side-chain-cleavage enzyme by ketoconazole with an ID_{50} of $\sim 0.3\ \mu M$ (Fig. 5), which is similar to that in rat adrenal cells ($ID_{50} \sim 0.5\ \mu M$) [5].

The non-cytochrome P-450 dependent enzymes 5-ene-3β-hydroxysteroid dehydrogenase, steroid isomerase, and 17β-hydroxysteroid dehydrogenase were not inhibited by ketoconazole. Also, the microsomal cytochrome P-450 enzyme, 17-hydroxylase was not inhibited. Previous data in adrenal glands [5] also showed that ketoconazole did not inhibit another microsomal cytochrome P-450 enzyme, 21-hydroxylase. In contrast, there was a clear inhibition of the microsomal cytochrome P-450 enzyme C-17,20-lyase.

Nakajin *et al.* have purified a single protein from neonatal pig testis possessing both 17α-hydroxylase and C-17,20 lyase activities [20]. Also, a variety of inhibitors affected both activities to the same extent. The authors concluded that a single cytochrome P-450 enzyme catalyzed both reactions. The current findings of inhibition of only one of these two enzymatic conversions in crude rat microsomal preparation might provide a valuable probe of the mechanism of the enzyme system.

The sensitivity to ketoconazole of the C-17,20-lyase ($ID_{50} \sim 12\ \mu M$) was much decreased compared to that of cholesterol-side-chain-cleavage enzyme ($ID_{50} \sim 0.3\ \mu M$). These data suggest that inhibition of cholesterol-side-chain-cleavage enzyme might be the more critical step in ketoconazole blockade of steroidogenesis. However, Santen *et al.* noted accumulation of 17-hydroxyprogesterone in patients receiving ketoconazole suggesting a significant action at C-17,20 lyase *in vivo* [6]. Ketoconazole may also importantly inhibit steps proximal to cholesterol in human cells. In a recent study, Buttke and Chapman [21] showed that ketoconazole inhibited the microsomal cytochrome P-450 enzyme, C-14 lanosterol demethylase in humans and murine lymphocytes. Furthermore, the ketoconazole ID_{50} for this step, $\sim 0.5\ \mu M$, is similar to that of cholesterol-side-chain-cleavage enzyme and total testosterone production in the current study.

Patients treated with routine ketoconazole dosages attain peak blood levels of 4–40 μM of drug [22], which exceeds the ID_{50} for inhibition of both cholesterol-side-chain-cleavage and C-17,20 lyase. In fact, multiple points of inhibition by ketoconazole probably contribute to the observed dramatic reduction in testosterone levels in male patients [3].

Although our experiments in this paper focus on antifungal imidazoles, other imidazole drugs could potentially cause similar steroidogenic inhibition [8, 9]. In recent studies, etomidate, an imidazole anesthetic agent, was found to lower cortisol levels in patients [23–25]. We have recently demonstrated in rat adrenal cells *in vitro* that the site of etomidate action is inhibition of both cholesterol-side-chain-cleavage and 11-hydroxylase enzymes [25]. Thus, in addition to imidazole antifungals, other imidazole drugs also affect steroidogenic cytochrome P-450 enzymes in patients.

The data presented in this paper support the hypothesis previously raised [2, 3, 5] that the imidazole antifungal drugs may be useful as steroid synthesis inhibitors. The *in vivo* studies indicate that adequate concentrations can be achieved for this purpose in patients. Endocrine side effects of ketoconazole are rare at the usual antifungal dosage, probably because clinically important inhibition of steroid production is transient, coinciding with peak plasma levels of drug [2, 3]. However, recent reports using high dose ketoconazole administered several times per day, indicate that this drug is useful in the treatment of Cushing's syndrome [26, 27]. Currently trials are also underway to use high dose ketoconazole therapy to inhibit testosterone production in patients with advanced prostate cancer [28]. The current findings extend the scope both of the number of drugs that may be employed to achieve steroidogenic inhibition as well as the number and selection of target cytochrome P-450 enzymes in multiple organs that might be amenable to blockade. The data presented raise the possibility of synthesizing relatively more specific antagonists designed to preferentially block a specific P-450 enzyme pathway. The utility of such an approach in prostate cancer and Cushing's syndrome are most obvious, but the importance and ubiquity of cytochrome P-450 enzymes leads us to predict other possible therapeutic applications for cytochrome P-450 inhibitors.

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