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Dermatophyte-Hormone Relationships: Characterization of Progesterone-Binding Specificity and Growth Inhibition in the Genera *Trichophyton* and *Microsporum*

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We reported previously that *Trichophyton mentagrophytes* contains a cytoplasmic macromolecule which specifically binds progesterone. Progesterone is also an effective inhibitor of growth of the fungus. We report here studies which characterize more fully the specific binding properties and the functional responses of *T. mentagrophytes* and taxonomically related fungi to a series of mammalian steroid hormones. Scatchard analysis of [^{3}H]progesterone binding in both the + and − mating types of *Arthroderma benhamiae* and in *Microsporum canis* revealed a single class of binding sites with approximately the same affinity as that in *T. mentagrophytes* (K_d, 1×10^{-7} to 2×10^{-7} M). *Trichophyton rubrum* had a protein with a higher binding affinity (K_d, 1.6×10^{-8} M). Characterization of the [^{3}H]progesterone-binding sites in *T. mentagrophytes* showed the binder to be a protein which was destroyed by trypsin and heating to 56°C. Previous examination of the steroid-binding specificity in *T. mentagrophytes* had demonstrated that deoxycorticosterone (DOC) and dihydrotestosterone (DHT) were effective competitors for [^{3}H]progesterone binding. Expansion of this study to include other competitors revealed that R5020 (a synthetic progestin), androstenedione, and dehydroepiandosterone possessed relative binding affinities which were 20, 11, and 9% of that of progesterone, respectively. Other ligands tested were less effective. Competition studies for the binder in *M. canis* resulted in similar findings: DOC and DHT were effective competitors for [^{3}H]progesterone binding. The growth of *A. benhamiae* + and −, *M. canis*, and *T. rubrum* were all inhibited by progesterone in a dose-responsive manner, with 50% inhibition achieved at concentrations of 9.8×10^{-6}, 1.2×10^{-5}, 1.5×10^{-5}, and 2.7×10^{-6} M, respectively. In addition, in the same rank order as specific progesterone binding, DOC and DHT were less effective inhibitors of growth of *M. canis* and *T. rubrum*. In conclusion, these results indicate that specific binding of progesterone appears to be a general phenomenon in dermatophytic fungi. Moreover, other steroid hormones and analogs inhibit growth in approximately the same rank order in which they displace [^{3}H]progesterone from the fungal binders. These results further substantiate our hypothesis that the binder acts as the molecular mediator of the inhibitory effects exerted by steroid hormones on the growth of these fungi. The significance of these responses to an aspect of the biology or pathogenesis (or both) of these organisms remains to be elucidated.

Recent studies of the interactions of mammalian steroid hormones with fungi have demonstrated specific steroid-binding moieties in several fungal genera (4, 6–9, 11–13, 18, 19). In addition, treatment of fungal cultures with steroid hormones has resulted thus far in the demonstration of steroid-modulated responses in three pathogenic fungi: *Paracoccidioides brasiliensis* (9, 13), *Coccidioides immitis* (3, 11), and *Trichophyton mentagrophytes* (18). For each, it has been hypothesized that the hormone-induced responses are mediated via the specific steroid-binding proteins. Furthermore, it has been postulated that the effect of the hormone may be related to the modulation of the pathogenesis of these fungi (3, 9, 11, 13). Our initial studies of *T. mentagrophytes* indicated that cytoplasmic extracts prepared from the organism contained a macromolecular component, presumably a protein, capable of specifically binding [^{3}H]progesterone (18). We shall refer to this molecule as progesterone-binding protein (PBP). Two other steroid ligands, deoxycorticosterone (DOC) and dihydrotestosterone (DHT), competed for specific [^{3}H]progesterone binding to PBP with moderate potency (18). In addition, progesterone inhibited the growth of *T. mentagrophytes* in a dose-dependent manner (18). DOC and DHT also inhibited the growth of *T. mentagrophytes* in the same rank order as their binding activity (i.e., progesterone > DOC > DHT) (18).

We report here the results of further studies which characterize more fully the specific progesterone-binding properties as well as the functional response of *T. mentagrophytes* to a variety of mammalian steroids. In addition, other related fungi, *Arthroderma benhamiae* (a teleomorph of *T. mentagrophytes*), *Trichophyton rubrum*, and *Microsporum canis*, were studied. We present evidence that the specific binding of progesterone by cytosolic extracts as well as the growth-inhibiting effect of progesterone in vitro appears to be general phenomena exhibited by dermatophytic fungi.

MATERIALS AND METHODS

Materials. [^{3}H]progesterone (56 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, Ill.). Radioinert steroids were obtained from Steraloids (Wilton, N.H.), with the exception of the synthetic progesterone, R5020 (prome-

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gestone), which was obtained from Dupont, NEN Research Products (Wilmington, Del.). Reagents for extracting and measuring ATP were purchased from 3M (St. Paul, Minn.) and LKB (Turku, Finland).

Fungi. Clinical isolates of *T. mentagrophytes* (isolate 5), *T. rubrum*, and *M. canis* kept in our stock culture collection for 2 to 5 years were grown on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants, stored at 4°C, and transferred yearly. During the course of this study, subcultures were maintained on Sabouraud dextrose agar slants at ambient temperature and transferred monthly. Cultures with aberrant macroscopic or microscopic morphology (i.e., reduction of microconidia formation, changes in colonial morphology, etc.) were discarded, and fresh subcultures were made from a long-term stock culture. The mating types of *T. mentagrophytes*, *A. benhamiae* + (RV 30000) and − (RV 30001), were the kind gift of C. DeVroey (Instituut voor Tropische Geneeskunde Prins Leopold, Antwerp, Belgium) and were maintained similarly.

Preparation of cytosol. Cytosol was prepared from *T. mentagrophytes*, *T. rubrum*, *M. canis*, and *A. benhamiae* cultures as described previously (18). In brief, 250 ml of Sabouraud dextrose broth (Difco) was inoculated with a suspension of conidia and mycelial fragments from 2- to 4-week-old slant cultures. Cultures were incubated for 7 days on a gyratory shaker (150 rpm) at room temperature, harvested by filtration, and washed with cold saline and cold homogenization buffer (250 mM sucrose, 1.5 mM EDTA, 10 mM Tris, 12 mM monothioglycerol, 10 mM sodium molybdate plus 10% [vol/vol] glycerol, pH 7.8). The mycelial mass was suspended in an equal volume of the homogenization buffer and disrupted mechanically as described previously (18). Cell debris and glass beads were removed by an initial low-speed centrifugation (1,200 × *g*, 4 min, 4°C). These supernatants were recentrifuged (204,000 × *g*, 30 min, 4°C), and cytosols were recovered as the resultant supernatants. Cytosol protein concentrations were determined by the Coomassie dye-binding technique (1).

Steroid binding. To determine total binding, cytosol was incubated with various concentrations of [^{3}H]progesterone at 0°C for 3 h. Nonspecific binding was assessed by concurrent incubation of replicate samples with a 500-fold molar excess of radioinert progesterone. The final sample volume for all experiments was 0.5 ml. Bound hormone was separated from free hormone by the addition of 0.5 ml of dextran-coated charcoal (0.5% Norit A [J. T. Baker Chemical Co., Phillipsburg, N.J.], 0.05% dextran) in homogenization buffer minus sucrose and incubated for an additional 12 min at 0°C. The samples were centrifuged (3,300 × *g*, 7 min, 4°C), and 0.25-ml portions of each supernatant were assayed for radioactivity by liquid scintillation counting. Specific binding was calculated by subtracting nonspecific binding from total binding. Specific binding data were subjected to Scatchard analysis (17) or assessed for percent reduction of specific binding by radioinert-competitor analysis.

Physical-chemical studies. Cytosol was prepared from *T. mentagrophytes* as described above. To assess the thermal stability of the binder, unbound cytosol was incubated at 0°C, 37°C, or 56°C for 30 min prior to determination of specific binding (6). Susceptibility of the binder to enzymatic degradation was determined by incubation of unbound cytosol for 30 min at 37°C in the presence of RNase A (100 μg/ml; type 1-AS; Sigma Chemical Co., St. Louis, Mo.) or trypsin (100 μg/ml; type III; Sigma) (6). DNase I (100 μg/ml; code DP; Worthington Biochemical Products, Malvern, Pa.) and phospholipase A_2 (100 μg/ml; from porcine pancreas; Sigma) were tested in the presence of 6 mM $MgCl_2$ and 2 mM $CaCl_2$, respectively (6). After each of the various treatments, samples were placed at 0°C and specific [^{3}H]progesterone binding was determined by the dextran-coated charcoal method.

Effect of steroids on in vitro growth of dermatophytic fungi. Growth was determined by bioluminescence assays of fungal ATP (10, 18) by using a luminometer (LKB). In brief, organisms were grown in a synthetic liquid medium (5) at 30°C for 8 days at 150 rpm on a gyratory shaker and then homogenized; the optical density was adjusted to an A_{450} of 0.6, and cells were diluted 1:25 in medium. Appropriate dilutions of progesterone or other steroids were made in medium plus carrier (1% ethanol for *T. mentagrophytes* and *T. rubrum* or 1% dimethyl sulfoxide [DMSO] for *M. canis*). Tubes containing 0.5 ml of mycelial suspension and 0.5 ml of medium with steroid or ethanol or DMSO control (final ethanol or DMSO concentration of 0.5%) were incubated at 30°C (18). ATP determinations were done in triplicate at time zero and every 24 h thereafter with commercially available reagents (NRB [nucleotide releasing agent for microbial ATP], 3M.; ATP Monitoring Kit, LKB). ATP concentrations were calculated by using internal standards of ATP.

Statistics. Data were analyzed by Student's *t* test and the Pearson correlation coefficient where indicated.

RESULTS

[^{3}H]progesterone binding. To determine whether related dermatophytic fungi possessed cytosolic [^{3}H]progesterone binding sites similar to PBP in *T. mentagrophytes*, we examined *T. rubrum*, *M. canis*, and *A. benhamiae* + and − mating strains. All exhibited specific and saturable [^{3}H] progesterone binding. Nonspecific binding was linear and generally less than 30% of the total binding, with the exception of *T. rubrum*, in which nonspecific binding approached 70%. To characterize these binding sites, saturation analysis and Scatchard plots were done for each cytosol. The results presented in Fig. 1 are representative Scatchard analyses from *A. benhamiae* + and − strains. The data obtained from all experiments with these organisms are presented in Table 1 along with data reported previously (18) for *T. mentagrophytes* for comparison. All organisms demonstrated a single class of binding sites with characteristics similar to the PBP in *T. mentagrophytes*.

The physical and biochemical characteristics of the [^{3}H]progesterone binder in *T. mentagrophytes* were assessed by examination of thermal stability and susceptibility to enzymatic degradation (Fig. 2). When compared with the usual 0°C incubation temperature, the binder was partially labile at 37°C. However, substantial binding remained at 37°C, whereas the binding essentially was destroyed by 30 min of treatment at 56°C. The binder was destroyed by treatment with trypsin but not by treatment with other enzymes.

Steroid specificity of the [^{3}H]progesterone-binding site. Because all organisms tested exhibited a similar [^{3}H]progesterone binder, we focused our efforts on further characterization of PBP in *T. mentagrophytes*. In our previous study, it was determined that DOC and DHT were good competitors for [^{3}H]progesterone binding in *T. mentagrophytes*, exhibiting 10 and 5% of the activity of progesterone, respectively (18). The specificity of the binding site was further examined in this study by determining the capacity of a variety of previously untested radioinert steroid hormones to compete for [^{3}H]progesterone binding (Fig. 3). As expected, progesterone proved to be the most effective competitor,

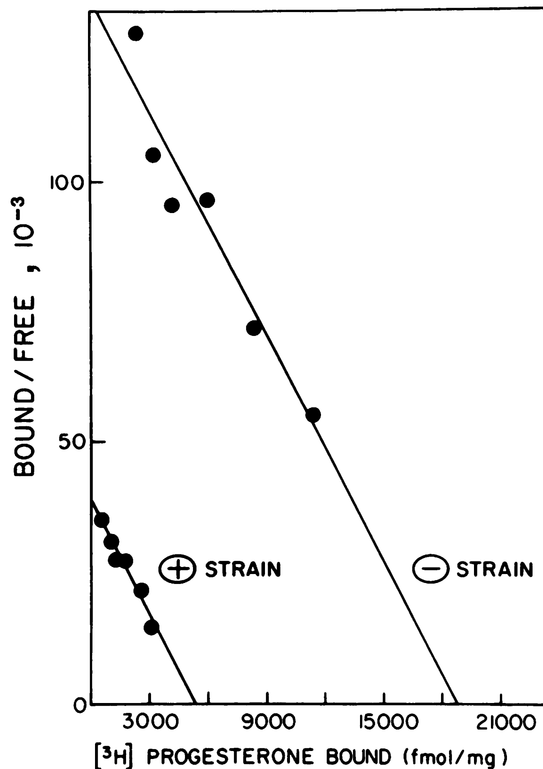

FIG. 1. Scatchard analysis of specific progesterone binding in *A. benhamiae* cytoplasmic extract. The dissociation constants in this study were 1.4×10^{-7} M for *A. benhamiae* + and 1.4×10^{-7} M for *A. benhamiae* −, and the binding capacities were 5,372 and 19,004 fmol/mg of cytosol protein, respectively ($r = -0.96$ for + and -0.94 for −). Bound/free, Bound [^{3}H]progesterone/free [^{3}H]progesterone.

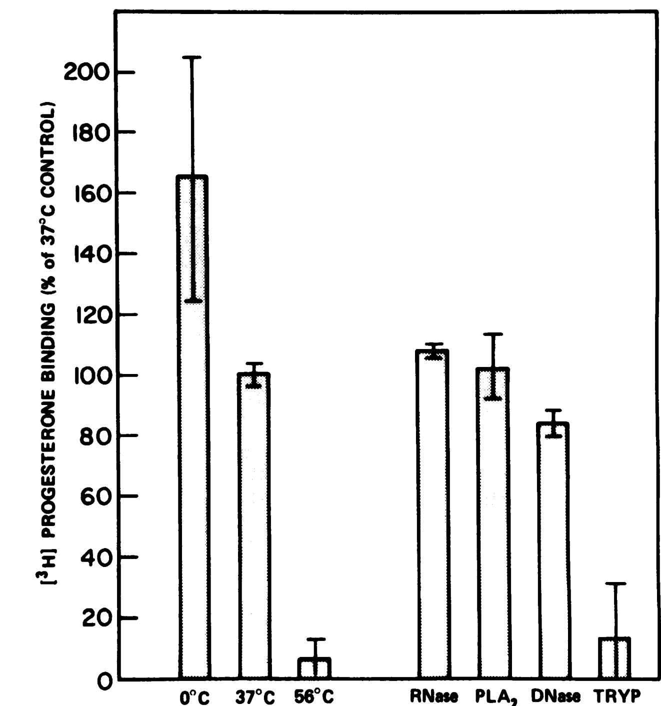

FIG. 2. Stability of the *T. mentagrophytes* binding site to thermal and enzymatic treatment. Cytoplasmic extract was treated at 0, 37, or 56°C alone or with various enzymes (100 μg/ml) at 37°C for 30 min. After treatment, [^{3}H]progesterone binding was assayed at 0°C for 3 h. The results are presented as the percentage of the binding in control samples held at 37°C. Abbreviations: PLA$_2$, phospholipase A$_2$; TRYP, trypsin.

inhibiting binding by 50% at a molar progesterone/[^{3}H]progesterone ratio of 1.3 under the conditions used. R5020 was the next best competitor, with a relative binding activity of 20% of progesterone. The relative binding activities of androstenedione and dehydroepiandosterone (DHEA) were 11 and 9%, respectively. Testosterone, 17αOH-progesterone, aldosterone, and 18-OH-DOC were all 3 to 5% as potent as progesterone. Dihydroandrosterone had negligible activity. The relative competitive potencies of all compounds tested, both in the prior study (18) and in the present study, were as follows: progesterone (100%) >> R5020 (20%) > androstenedione (11%) ≥ DOC (10%) ≥ DHEA (9%) > DHT (5%) = testosterone (5%) = 17αOH-progesterone (5%) > 18-OH-deoxycorticosterone (3%) = aldosterone (3%). Cortexolone, corticosterone, dexamethasone, cortisol, estradiol, and dihydroandrosterone had negligible competitive activity.

To ascertain whether the PBP in other dermatophytes was similar to the binding site in *T. mentagrophytes*, a competi-

TABLE 1. [^{3}H]progesterone binding in cytoplasmic extracts from various dermatophytes

Organism	K_d (10^{-8} M)[a]	N_{max} (fmol/mg of protein)[b]
T. mentagrophytes[c]	9.5 ± 2.4	4,979 ± 3,489
A. benhamiae +	16.2 ± 3.0	8,894 ± 4,980
A. benhamiae −	17.3 ± 4.0	14,385 ± 6,531
M. canis	19.0 ± 6.0	15,556 ± 4,301
T. rubrum	1.6 ± 0.5	196 ± 157

[a] Apparent dissociation constant.
[b] Maximum number of binding sites.
[c] Data derived from reference 18.

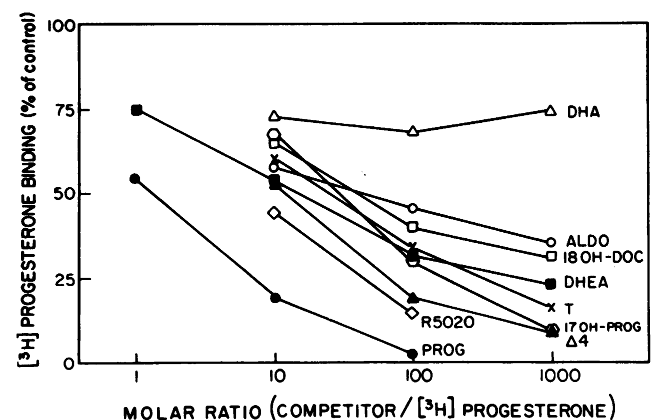

FIG. 3. Specificity of the fungal binder in *T. mentagrophytes* studies by competition analysis. Cytoplasmic extract was incubated for 3 h at 0°C with 1.3×10^{-7} M [^{3}H]progesterone with or without the indicated concentration of radioinert competitor. Binding in the absence of competitor was taken as 100%. Results are the means of at least four determinations. Abbreviations: PROG, progesterone; R5020, promegestone; Δ4, androstenedione; 17OH-PROG, 17-hydroxyprogesterone; T, testosterone; DHEA, dehydroepiandosterone; 18OH-DOC, 18-OH-deoxycorticosterone; ALDO, aldosterone; DHA, dihydroandrosterone.

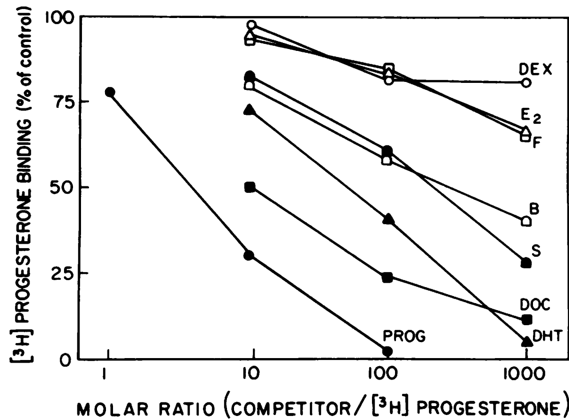

FIG. 4. Specificity of the fungal binder in *M. canis* as determined by competition analysis. Cytoplasmic extract was incubated for 3 h at 0°C with 1.3×10^{-7} M [³H]progesterone with or without the indicated concentration of radioinert competitor. Binding in the absence of competitor was taken as 100%. Results are the means of at least four determinations. Abbreviations: PROG, progesterone; S, deoxycortisol; B, corticosterone; F, cortisol; E_2, 17β-estradiol; DEX, dexamethasone.

tion analysis for specific progesterone binding was done with cytosol from *M. canis* (Fig. 4). Again, progesterone was the most potent competitor, inhibiting binding by 50% at a molar progesterone/[³H]progesterone ratio of 3.8 under the conditions used. Of the other compounds tested, DOC and DHT were the next most potent steroids, with relative binding activity values of 38 and 8%, respectively. The other steroids tested were much less active. In order, the relative potencies of all steroids tested in cytosol of *M. canis* were: progesterone (100%) >> DOC (38%) >> DHT (8%) > deoxycortisol (2%) = corticosterone (1%). Cortisol, estradiol, and dexamethasone were inactive at the concentrations tested. This pattern was similar to that obtained with *T. mentagrophytes* PBP.

Effect of hormones on fungal growth. In the previous study with *T. mentagrophytes*, steroid hormones which demonstrated binding activity for PBP were found to inhibit growth as determined by cellular ATP accumulation (18). Similar studies were done in the current investigation of the growth of both *T. rubrum* (Fig. 5A) and *M. canis* (Fig. 5B). Progesterone exhibited a strong inhibitory effect on the growth in vitro of both organisms. However, there was an apparent recovery from growth inhibition (escape) by both organisms which occurred after 24 h. In addition, both DOC and DHT, when tested at 6.5×10^{-5} M, inhibited the growth of *T. rubrum* (Fig. 5A) and *M. canis* (Fig. 5B) to a significant degree throughout the assay period (24, 48, and 72 h; $P < 0.05$). However, both DOC and DHT inhibited the growth of *T. rubrum* and *M. canis* to a lesser degree than did progesterone. Escape from growth inhibition was detected with these steroids as well. In dose-response studies, the concentrations of progesterone that inhibited the growth of *T. rubrum* and *M. canis* by 50% were determined to be 2.7×10^{-6} M and 1.5×10^{-5} M, respectively (Fig. 6). The potency of growth inhibition exhibited by progesterone, DOC, and DHT against *M. canis* followed the same rank order as did the binding potency. Although competition analysis of [³H]progesterone binding was not examined in *T. rubrum*,

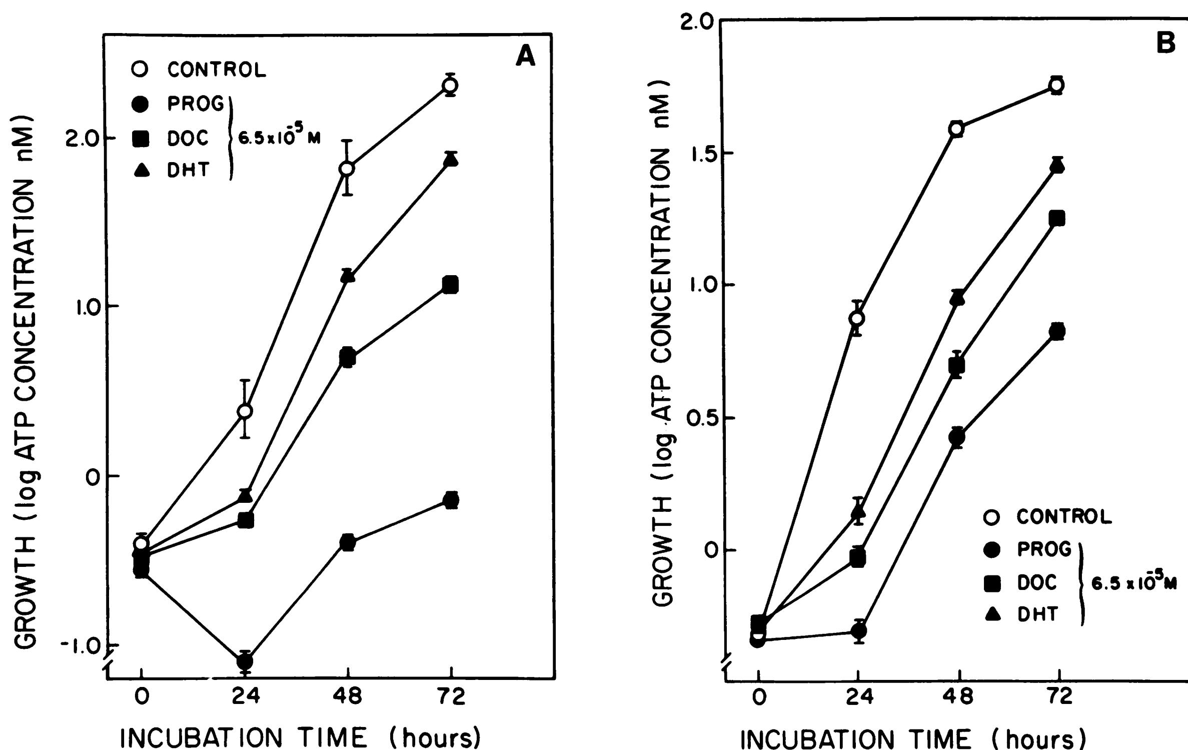

FIG. 5. Growth inhibition by various steroids. The growth of (A) *T. rubrum* and (B) *M. canis* was assessed by accumulation of cellular ATP measured at 24-h intervals in the presence of 6.5×10^{-5} M progesterone (PROG), DOC, DHT, or medium control. The data are expressed as $\log_{10}$ ATP concentration in nanomoles (± the standard deviation) of culture samples at 0, 24, 48, and 72 h.

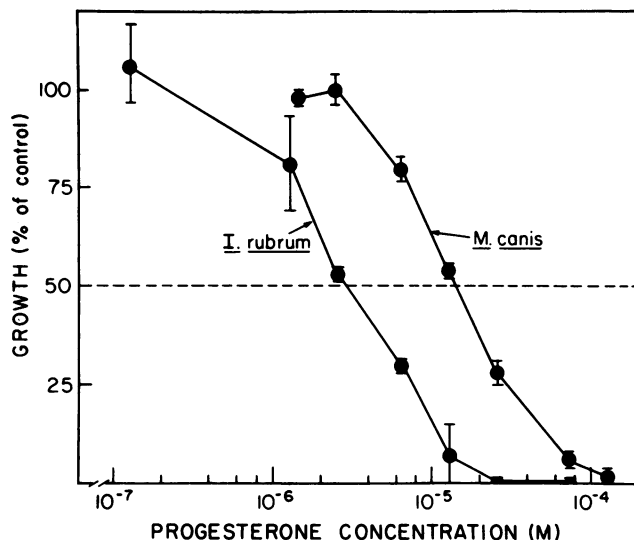

FIG. 6. Effects of various concentrations of progesterone on the growth of *T. rubrum* and *M. canis*. Data are expressed as percent growth (± standard deviation) compared with a concurrent control after 48 h of incubation. Fifty percent inhibition was achieved by progesterone at 2.7×10^{-6} M in *T. rubrum* and at 1.5×10^{-5} M in *M. canis*.

the growth inhibition data were in the same rank order as it was for both *M. canis* and *T. mentagrophytes*.

Previous studies of the effect of steroid hormones on *T. mentagrophytes* had indicated a strong correlation between binding potency for PBP and rank order of growth inhibition (18). These studies were expanded to include R5020 and DHEA, which were two of the best progesterone-binding competitors of the compounds tested (Fig. 3). R5020 and DHEA were found to be almost as potent as progesterone in inhibiting the growth of *T. mentagrophytes* (Table 2). Additional studies with *A. benhamiae* + and −, the mating types of some *T. mentagrophytes*, also demonstrated progesterone-mediated dose-dependent inhibition of growth (Table 2).

DISCUSSION

Proteins which bind vertebrate steroid hormones have been demonstrated to be present in a number of different fungi (4, 6–9, 11, 12, 18, 19). It has been proposed that these binding proteins represent primitive receptors which, as the molecular sites of action, mediate functional responses to mammalian steroid hormones (4, 6–9, 11, 18). Indeed, this would appear to be a possibility for several fungi that exhibit bioresponses to particular hormones (3, 13, 18). One example, *P. brasiliensis*, binds 17β-estradiol with high affinity and stereospecificity (9, 19). Functionally, estradiol blocks the mycelium-to-yeast morphogenic transition of *P. brasiliensis* in a dose-responsive manner (13). *T. mentagrophytes* possesses a binding moiety, which has been shown to be a thermolabile protein (Fig. 2) that specifically binds progesterone (18). In the presence of progesterone, the growth of the organism is inhibited in a dose-dependent manner (18). As part of an expanded study of the interactions of mammalian steroid hormones with *T. mentagrophytes*, we have investigated further both the binding properties and the functional effects of a variety of steroid hormones on several dermatophytic organisms.

TABLE 2. Comparison of growth-inhibitory potency of steroids for *T. mentagrophytes* and *A. benhamiae*

Organism	Steroid	ED_{50} (μM)[a]
T. mentagrophytes	Progesterone	6.7 ± 2.7
	R5020	7.0 ± 4.5
	DHEA	7.5 ± 4.8
A. benhamiae +	Progesterone	9.9 ± 3.0
A. benhamiae −	Progesterone	12.1 ± 2.5

[a] Concentration of steroid which inhibited growth to 50% of control growth. Values are the means ± standard deviations; the number of determinations was three, except for progesterone in *T. mentagrophytes* ($n = 13$).

We examined the possibility that one but not the other mating type of *A. benhamiae* might bind progesterone. Such a result could have potential relevance to sexual mating, as is the situation with *Achlya ambisexualis* (14–16). In that organism, female cells produce the steroidal compound antheridiol, which induces sexual differentiation by the male cells that is mediated via a specific receptor for antheridiol (14–16). However, an equivalent antheridiol receptor is not present in the female cells, nor do the female cells respond to antheridiol (15). Both mating types of *A. benhamiae*, + and −, bound progesterone with equal affinities, which were similar to that of *T. mentagrophytes* (Table 1) (18). Since both mating types possess a PBP, these findings do not appear to be similar to the *Achlya ambisexualis* system. However, these results do not exclude the possibility that either one or both types produce natural ligands involved with sexual differentiation which bind with much greater affinity than does the mammalian hormone progesterone. Ligands produced by the mating types may differ structurally from each other as well as from progesterone. It should be noted that in higher eucaryotes, both male and female possess receptors for both androgens and estrogens.

The presence of specific progesterone binding and a functional response to progesterone by *T. rubrum* and *M. canis* appears to indicate that binding as well as inhibition of growth are general properties of these taxonomically related fungi (Fig. 4, 5, and 6; Tables 1 and 2). In addition, the hormone-binding profile demonstrated for *M. canis* PBP correlated with the potency of growth inhibition exhibited by the steroid hormones (i.e., progesterone, the most active competitor for binding, was the most potent inhibitor of growth) (Fig. 4 and 5B). These data are in agreement with earlier data on *T. mentagrophytes* (18) in that the binding activity of a steroid hormone to PBP in this system corresponds with its capacity to inhibit growth, supporting the hypothesis that the functional effect is regulated by the PBP. It is of interest that an apparent relationship between the K_d of the binder and the relative concentration of progesterone that inhibits growth by 50% (50% effective dose [ED_{50}]) exists in these systems. The ED_{50} concentration for progesterone action in *T. rubrum* (2.7×10^{-6} M) is approximately 5- to 10-fold lower than the progesterone ED_{50} for *M. canis* (1.5×10^{-5} M). Similarly, the K_d for progesterone binding in *T. rubrum* (1.6×10^{-8} M) is of approximately 10-fold greater affinity than that of *M. canis* (K_d, 1.9×10^{-7} M). Compared with previous results obtained with *T. mentagrophytes* (ED_{50}, 5.5×10^{-6} M; K_d, 9.5×10^{-8} M) (18), it would appear that the higher the binding affinity for progesterone, the lower the ED_{50} of progesterone necessary to inhibit growth. The 100-fold differences between relative ED_{50} concentrations necessary for action and K_d values for binding are as yet unexplained but may be related to metabolism of added hormone or impeded entry of progesterone into intact cells

or both. Since *T. mentagrophytes* has been shown to convert progesterone to more polar endproducts (2; K. V. Clemons, E. P. Stover, G. Schär, D. Feldman, and D. A. Stevens, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, F55, p. 373), metabolism may be primarily responsible for the apparent 100-fold differences between the ED_{50} and the K_d concentrations.

Expansion of the binding specificity studies of *T. mentagrophytes* PBP revealed that progestins and androgenic compounds were the most active competitors for specific progesterone binding (Fig. 3). The mineralocorticoid DOC (21-hydroxyprogesterone), structurally related to progesterone, was also very potent. In addition, two of the most active competitors, R5020 and DHEA, were demonstrated to be potent inhibitors of growth when tested for functional activity. These results are in agreement with previous data and further support the hypothesis that growth inhibition is mediated via PBP (18). The synthetic progestin, R5020, has a high affinity for mammalian progesterone receptors but has been reported to be only 2% as effective as progesterone as a competitor for the corticosterone-binding protein of *Candida albicans* (6, 7). It should be noted that in the *C. albicans* system, corticosterone and progesterone bind with equal affinity to the same protein (6–8). However, unlike the situation with *C. albicans*, corticosterone is only 1% as effective as progesterone as a competitor for *T. mentagrophytes* PBP (18), whereas R5020 is an excellent competitor. Thus, these data demonstrate inherent differences between the binding proteins of *C. albicans* and *T. mentagrophytes*.

In summary, the results presented here demonstrate that progesterone and related steroids bind with high specificity to a cytoplasmic protein and that binding appears to be a general property of *T. mentagrophytes* and taxonomically related fungi. Furthermore, progesterone and related steroids that are capable of binding also exhibit the capacity to inhibit the growth of dermatophytes. These data support our hypothesis that the binder represents a putative receptor system which mediates a functional effect in the organism. The specific actions mediated by the steroid-binding proteins are not yet defined. Whether the responses are important to the biology of the organism (i.e., such as in sexual mating), represent a mechanism of morphogenic regulation, or are integral to pathogenesis (or some combination of all of these actions) remains to be elucidated.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. **72**:248–254.
2. **Capek, A., and A. Simek.** 1971. Effect of steroids on dermatophytes. Folia Microbiol. **16**:299–302.
3. **Drutz, D., M. Huppert, S. H. Sun, and W. L. McGuire.** 1981. Human sex hormones stimulate the growth and the maturation of *Coccidioides immitis*. Infect. Immun. **32**:897–907.
4. **Feldman, D., Y. Do, A. Burshell, P. Stathis, and D. S. Loose.** 1982. An estrogen-binding protein and endogenous ligand in *Saccharomyces cerevisiae*: possible hormone receptor system. Science **218**:287–298.
5. **Hoeprich, P. D., and P. D. Finn.** 1972. Obfuscation of the activity of antifungal antibiotics by culture media. J. Infect. Dis. **126**:353–361.
6. **Loose, D. S., and D. Feldman.** 1982. Characterization of a unique corticosterone-binding protein in *Candida albicans*. J. Biol. Chem. **257**:4925–4930.
7. **Loose, D. S., D. J. Schurman, and D. Feldman.** 1981. A corticosteroid binding protein and endogenous ligand in *Candida albicans* indicating a possible steroid-receptor system. Nature (London) **293**:477–479.
8. **Loose, D. S., D. A. Stevens, D. J. Schurman, and D. Feldman.** 1983. Distribution of a corticosteroid-binding protein in *Candida* and other fungal genera. J. Gen. Microbiol. **129**:2379–2385.
9. **Loose, D. S., E. P. Stover, A. Restrepo, D. A. Stevens, and D. Feldman.** 1983. Estradiol binds to a receptor-like cytosol binding protein and initiates a biological response in *Paracoccidioides brasiliensis*. Proc. Natl. Acad. Sci. USA **80**:7659–7663.
10. **Odds, F. C.** 1980. Laboratory evaluation of antifungal agents: a comparative study of five imidazole derivatives of clinical importance. J. Antimicrob. Chemother. **6**:749–761.
11. **Powell, B. L., D. J. Drutz, M. Huppert, and S. H. Sun.** 1983. Relationship of progesterone- and estradiol-binding proteins in *Coccidioides immitis* to coccidioidal dissemination in pregnancy. Infect. Immun. **40**:478–485.
12. **Powell, B. L., C. L. Frey, and D. J. Drutz.** 1984. Identification of a 17β-estradiol binding protein in *Candida albicans* and *Candida* (*Torulopsis*) *glabrata*. Exper. Mycol. **8**:304–313.
13. **Restrepo, A., M. E. Salazar, L. E. Cano, E. P. Stover, D. Feldman, and D. A. Stevens.** 1984. Estrogens inhibit mycelium-to-yeast transformation in the fungus *Paracoccidioides brasiliensis*: implications for resistance of females to paracoccidioidomycosis. Infect. Immun. **46**:346–353.
14. **Riehl, R. M., and D. O. Toft.** 1984. Analysis of the steroid receptor of *Achyla ambisexualis*. J. Biol. Chem. **259**:15324–15330.
15. **Riehl, R. M., and D. O. Toft.** 1985. Effect of culture medium composition of pheromone receptor levels in *Achlya ambisexualis*. J. Steroid Biochem. **23**:483–489.
16. **Riehl, R. M., D. O. Toft, M. D. Meyer, G. L. Carlson, and T. C. McMorris.** 1984. Detection of a pheromone-binding protein in the aquatic fungus *Achlya ambisexualis*. Exp. Cell Res. **153**:544–549.
17. **Scatchard, G.** 1949. The attractions of protein for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
18. **Schär, G., E. P. Stover, K. V. Clemons, D. Feldman, and D. A. Stevens.** 1986. Progesterone binding and inhibition of growth in *Trichophyton mentagrophytes*. Infect. Immun. **52**:763–767.
19. **Stover, E. P., G. Schär, K. V. Clemons, D. A. Stevens, and D. Feldman.** 1986. Estradiol-binding proteins from mycelial and yeast-form cultures of *Paracoccidioides brasiliensis*. Infect. Immun. **51**:199–203.