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Antagonism of Estrogen-Induced Prolactin Release by Dihydrotestosterone[1]

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ABSTRACT

Previous work from our laboratory has demonstrated that progesterone can inhibit estrogen-induced prolactin release in female rats. Since androgens have been reported to mimic progesterone actions in certain systems, and to antagonize estrogen action in rat uteri, the purpose of this study was to determine whether androgens, like progestins, can inhibit estrogen-induced prolactin release. The ovariectomized (26 days of age) immature rat was used as the model for analysis of this question. Dihydrotestosterone (DHT) was chosen to be used throughout the study since it does not undergo aromatization to estrogens. In response to estradiol exposure (2 μg/rat), prolactin release reached peak values at 12 h and returned to control levels by 24 h. A second injection of estradiol 13 h after its initial injection stimulated a second increase in serum prolactin at 25 h. A single injection of DHT (0.8 mg/kg BW) 1 h before the second estradiol injection blocked the increase in serum prolactin. DHT had no effect on basal serum prolactin levels. The DHT inhibition of estrogen-induced prolactin release required estrogen priming. A dose dependency for the DHT effect was demonstrated, with low doses effective and high doses ineffective, in inhibiting estrogen action. This effect of DHT seemed to be androgen receptor-mediated, since flutamide blocked the effect. However, the possibility of progestin receptor mediation could not be ruled out since RU486 also blocked DHT's effect. Flutamide was also effective in blocking progesterone's inhibition of estrogen-induced action. This is perhaps consistent with an overlap of activities in androgens and progestins reported by several investigators. These observations indicate that the role of androgens in a variety of experimental as well as clinically relevant situations needs to be explored not only in terms of direct action but also as modifying estrogen action.

INTRODUCTION

Previous work from our laboratory has shown that progesterone administration to estrogen-primed ovariectomized immature rats results in a rapid decrease in nuclear estradiol binding in the anterior pituitary (Smanik et al., 1983). This progesterone-induced decrease occurs selectively in occupied pituitary nuclear estradiol receptors (Fuentes et al., 1988) and is accompanied by a loss of estrogen action, such as estradiol-induced progesterone receptor synthesis (Calderon et al., 1987) and prolactin release (Brann et al., 1988). Since androgens have been reported to bind to progesterone receptors (Jänne and Bardin, 1984), mimic progestin actions in certain systems (Bardin et al., 1984), and also to decrease nuclear estradiol binding in the anterior pituitary of ovariectomized female rats (Keefer et al., 1987), it was of interest to examine whether androgens, like progesterone, could antagonize estradiol-induced prolactin release.

Antagonistic actions of androgens upon estrogen effects have been reported previously in uteri (Tran and Gibbons, 1983), in rat pituitary tumor (GH_3) cells (Haug, 1979), and in human breast cancer cells (Poulin, 1988). It is well recognized that prolactin is an important component in the reproductive process and in the control of growth and secretions of breasts. Thus androgen inhibition of estradiol-induced prolactin release may have important implications. Furthermore, androgens are important regulators of gonadotropin secretion and it was of interest to determine if their effects are only direct effects or whether they can modify estrogen action. Dihydrotestosterone (DHT) was chosen to be used throughout the study since it does not undergo aromatization to estrogens.

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MATERIALS AND METHODS

Animals

Immature, virus-free, female Holtzman rats (Madison, WI) were obtained at 26 days of age and were bilaterally ovariectomized on the same day under ether anesthesia. They were maintained in air-conditioned rooms with a 14L:10D cycle (lights on at 0500 h; off at 1900 h) and were given water and rat chow ad libitum. In all experiments, the rats were killed by decapitation and the trunk blood was collected. After clotting for 12 h at 4°C, the blood was centrifuged at 3000 rpm for 30 min at 4°C, and serum was separated and stored at –20°C for subsequent radioimmunoassay of prolactin. The protocols for the various experiments in this study were as follows.

Protocol A. To determine the effect of DHT on basal prolactin secretion, animals were ovariectomized at 26 days of age, and 48 h later (0900 h) were administered a single injection of estradiol (2 μg/rat). Twelve hours later (2100 h), either vehicle (controls) or DHT was administered i.p., and all animals were killed 24 h after estrogen (0900 h) administration for serum prolactin measurements.

Protocol B. To determine the effect of DHT on estrogen-induced prolactin secretion in non-estrogen-primed rats, ovariectomized rats (28 days old) were administered either vehicle (controls) or DHT at 2100 h, and 1 h later, a 2-μg dose of estradiol was administered. The animals were killed 12 h later (1000 h) for serum prolactin measurements.

Protocol C. To determine the effect of DHT on estradiol-induced prolactin secretion in estrogen-primed rats, 28-day-old ovariectomized rats were administered two injections of estradiol (2 μg/rat) 13 h apart (0900 h and 2200 h). One hour prior to the second estrogen injection, either vehicle or DHT was administered, and the animals were killed 12 h after the second estrogen injection (1000 h) for serum prolactin measurements. In the flutamide (α-α-α-trifluoro-2-methyl-4′-nitro-m-propionotoluidide) experiments, flutamide (5 mg/rat) was administered in a vehicle of propylene glycol i.p. 1 h before DHT or progesterone administration (2 h before the second estradiol injection). In the RU486 (17β-hydroxy-11β-[4-dimethylaminophenyl]-17α-[prop-1-ynyl]-estra-4,9-diene-3-one) experiments, RU486 (200 μg/rat) was administered i.p. in a vehicle of ethylene glycol 1 h before progesterone or DHT administration (2 h before the second estradiol injection). The animals were killed 12 h after the second estradiol injection by decapitation, and trunk blood was analyzed for serum prolactin content.

Radioimmunoassay of Prolactin

The concentration of prolactin in serum samples were measured by a double-antibody radioimmunoassay method as described by Rao and Mahesh (1986), using the first antibody for NIAMDD-rProlactin S-9 [rabbit] and purified hormone and standard obtained from NIAMDD. The purified hormone was iodinated with ^{125}I by the chloramine-T method (Bolton, 1977). The second antibody (goat anti-rabbit antiserum) was purchased from Arnell Inc., Brooklyn, NY. The assay was linear at 0.01–10 ng/tube for prolactin. With 50-μl samples, the intra- and interassay variabilities as determined by analysis of replicate serum pool samples were 7% and 11%. Prolactin levels are expressed in terms of NIAMDD-RP-3 standard.

Statistical Analysis

The results given in the text are expressed as means ± SEM. The differences between the experimental groups were analyzed by one-way analysis of variance, and $p<0.05$ was considered significant.

RESULTS

The model used to determine androgen effect upon estradiol-induced prolactin release was developed previously by us to examine progesterone modulation of estradiol-induced prolactin release (Brann et al., 1988). The model consisted of two i.p. injections of 2 μg of estradiol to immature ovariectomized rats administered 13 h apart followed by the measurement of serum prolactin 25 h after the first estradiol injection. The two-estradiol-injection model also ensures the presence of an adequate number of androgen receptors in the anterior pituitary (Handa et al., 1987).

To determine the effect of DHT on estrogen-induced prolactin release, immature ovariectomized rats were administered either vehicle or 0.8 mg/kg BW of DHT 1 h before the second injection of 2 μg of estradiol. The animals were killed 13 h later for measurement of serum prolactin levels (Protocol C). The results in Figure 1 show that the administration of DHT 1 h before the second injection of estradiol significantly

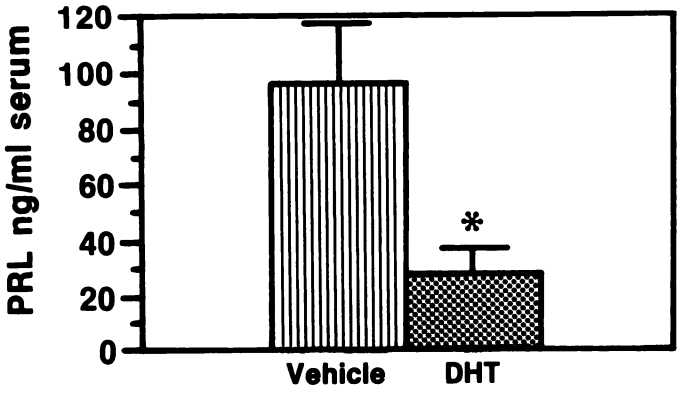

FIG. 1. Effect of dihydrotestosterone *(DHT)* on estrogen-induced prolactin *(PRL)* release when administered 1 h before the second estrogen injection. Two injections of 2 μg of estradiol were administered to ovariectomized immature rats at 0 h and 13 h. Controls received vehicle 1 h before the second estradiol injection, whereas the DHT group received 0.8 mg/kg BW of DHT 1 h before the second estradiol injection. Serum prolactin levels were measured 12 h after the second estradiol injection. *$p<0.05$.

attenuated ($p<0.05$) the estradiol-induced prolactin release.

To determine whether DHT could affect basal serum prolactin levels, DHT was administered 12 h after estrogen injection and the animals were killed at the 24-h time period. This was essentially the same experimental design shown in Figure 1, except that the second estradiol injection was omitted (Protocol A). As shown in Figure 2, prolactin levels 24 h after the estradiol injection were basal and DHT had no significant effect

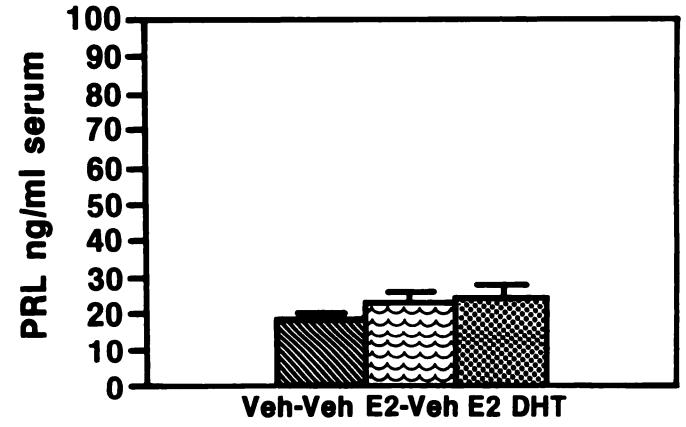

FIG. 2. Effect of dihydrotestosterone *(DHT)* on basal prolactin levels in estrogen-primed, ovariectomized immature rats. One injection of 2 μg estradiol in vehicle (E_2-Veh) was administered to ovariectomized, immature rats at Time 0. Controls *(Veh-Veh)* received vehicle at 12 h, whereas the DHT group (E_2-DHT) received 0.8 mg/kg BW DHT. The animals were killed at 24 h. There was no significant differences in serum PRL levels between the groups. The *figure* also shows serum PRL levels in ovariectomized immature rats that received only vehicle.

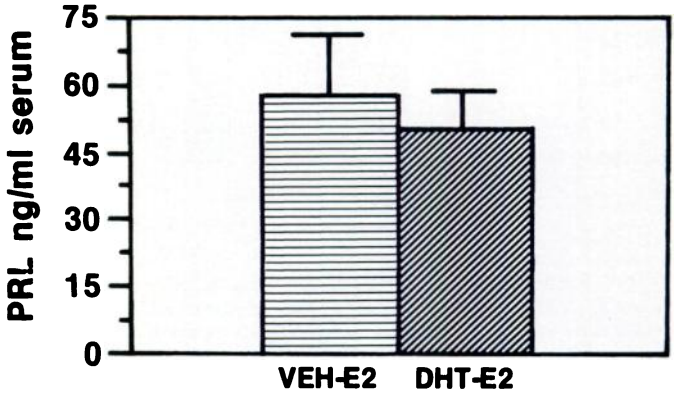

FIG. 3. Effect of dihydrotestosterone *(DHT)* on estrogen-induced prolactin *(PRL)* release in non-estrogen-primed rats. Ovariectomized immature rats were administered vehicle (controls, *VEH*) or *DHT* (0.8 mg/kg BW) 1 h before a 2 μg injection of estradiol (E_2); animals were killed 12 h after the estrogen injection.

on serum prolactin levels.

To determine whether DHT inhibition of estrogen-induced prolactin release required estrogen priming, an experiment was performed in which vehicle or DHT was injected 1 h before 2-μg estradiol was administered (Protocol B). As shown in Figure 3, DHT had no effect on the estrogen-induced prolactin release in non-estrogen-primed animals, whereas, in estrogen-primed animals, DHT significantly attenuated estradiol-induced prolactin release (Fig. 1).

To establish whether DHT inhibition of estrogen-induced prolactin release was dose dependent, an experiment was carried out using 0.4 mg/kg BW, 0.8 mg/kg BW, 1.6 mg/kg BW, 3.2 mg/kg BW, and 10 mg/kg BW doses of DHT. The protocol for this experiment is outlined in Protocol C. As shown in Figure 4, the 0.4 mg/kg BW, 0.8 mg/kg BW, and the 1.6 mg/kg BW

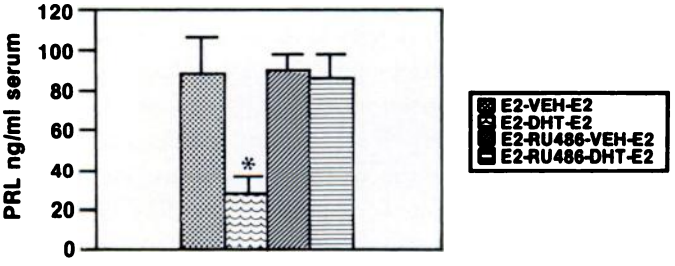

FIG. 4. Effect of different doses of dihydrotestosterone (DHT) on estrogen-induced prolactin *(PRL)* release. Two injections of 2 μg of estradiol (E_2) were administered to ovariectomized immature rats at 0 h and 13 h. Controls received vehicle 1 h before the second estradiol injection, whereas the DHT groups received one of the following doses of DHT 1 h before the second estradiol injection: 0.4 mg/kg BW, 0.8 mg/k BW, 1.6 mg/kg BW, 3.2 mg/kg BW, or 10 mg/kg BW. Serum PRL levels were measured 12 h after the second estradiol injection. $p<0.05$.

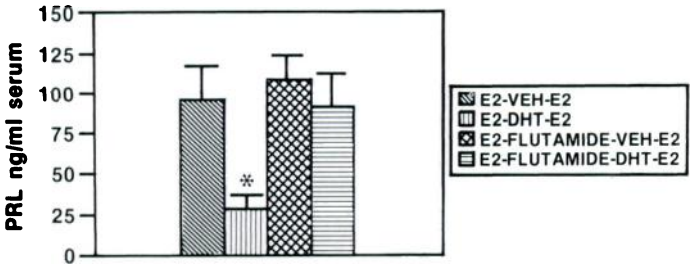

FIG. 5. Effect of the antiandrogen *flutamide* on the ability of dihydrotestosterone to *(DHT)* to inhibit the estrogen-induced prolactin *(PRL)* release. The model is the same as in *Figure 1*, except that two groups of animals received flutamide (5 mg/rat) 2 h before the second estrogen injection. One hour before the second estrogen injection, animals received either vehicle *(VEH)* or *(DHT)* (0.8 mg/kg BW). Finally, all animals received 2 μg of estradiol *(E_2)* 1 h later, and serum PRL levels were measured 12 h after the second estradiol injection.

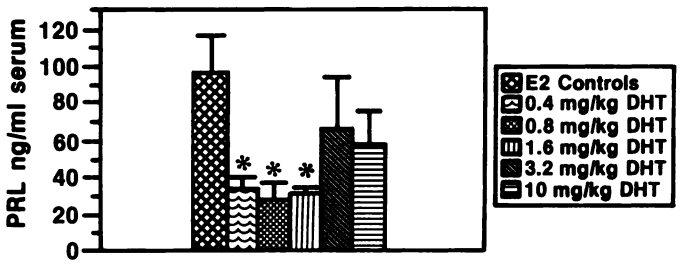

FIG. 6. Effect of the antiprogestin *RU486* on the ability of dihydrotestosterone *(DHT)* to inhibit the estrogen-induced prolactin *(PRL)* release. The model is the same as in Figure 1, except that two groups of animals received RU486 (200 μg/rat) 2 h before the second estrogen injection. One hour before the second estrogen injection, animals received either vehicle *(VEH)* or dihydrotestosterone (0.8 mg/kg BW). Finally all animals received 2 μg of estradiol *(E_2)* 1 h later, and serum PRL levels were measured 12 h after the second estradiol injection.

doses significantly attenuated the estrogen-induced prolactin release, whereas the higher doses of DHT, 3.2 mg/kg BW and 10 mg/kg BW, showed a much greater variability in effect and were not significantly different from the controls.

To determine whether the action of DHT in the attenuation of estrogen-induced prolactin release was an androgen-receptor-mediated event, the antiandrogen, flutamide (5 mg/rat), was administered 1 h prior to the injection of DHT (2 h before the second estradiol injection) (Protocol C). The injection of flutamide and vehicle instead of DHT served as a control. Flutamide by itself appeared to have no effect on estrogen-induced prolactin release (Fig. 5); however, the administration of flutamide 1 h before DHT prevented the DHT attenuation of estrogen-induced prolactin release.

To determine whether the action of DHT was a progesterone receptor-mediated event, the antiprogestin, RU486 (200 μg/rat), was administered 1 h prior to the injection of DHT (2 h before the second estradiol injection) (Protocol C). The injection of RU486 and vehicle instead of DHT served as a control. RU486, like flutamide, had no effect on estradiol-induced prolactin release when administered by itself (Fig. 6); however, RU486 administered 1 h before DHT completely blocked the DHT action of inhibiting estrogen-induced prolactin release (Fig. 6). Since progestins have also been reported to be able to use androgen receptors for their actions (Bardin et al., 1984), we designed an experiment where the ability of flutamide or RU486 to block progesterone's attenuation of estrogen-induced prolactin release was tested. The protocol was the same for the flutamide and RU486 experiments described in Figures 5 and 6, except progesterone (0.8 mg/kg BW) was used instead of DHT. As shown in Figure 7, progesterone significantly inhibited the estrogen-induced prolactin release, and both flutamide and RU486 prevented this effect of progesterone.

DISCUSSION

The overall objectives of this study were to determine whether androgens, like progestins, could alter or interfere with estrogen action in the pituitary. Estrogen-induced prolactin secretion was chosen as an indicator of estrogen action because (1) estrogens are known to be potent stimulators of prolactin release in the adult as well as in the immature rat (Chen and Meites, 1970; Ojeda and McCann, 1974; Andrews and Ojeda, 1977) and (2) because progestin inhibition of estrogen-induced prolactin release had been demonstrated by our laboratory previously (Brann et al., 1988).

In agreement with previous reports (Dohler et al., 1978; Parrot and Hills, 1979; Labrie et al., 1980), we found that DHT did not alter basal serum prolactin levels (Fig. 2); however, it did antagonize estrogen-induced prolactin release (Fig. 1).

DHT was ineffective in inhibiting estrogen-induced prolactin secretion when estrogen priming was omitted (Fig. 3). That estrogen priming is required for androgen action has been reported in chick oviducts (Tokarz et al., 1979). Estrogen pretreatment in this tissue results in inducement of androgen receptors and a concomitant return of androgen action (Tokarz et al., 1979). Similarly, estrogen priming in dog prostate results in inducement of androgen receptors and significant enhancement of androgen action (Moore et al., 1979).

Androgen receptor levels in the anterior pituitary of rats are known to decrease post-ovariectomy and to fluctuate during the cycle (Handa et al., 1986, 1987), perhaps suggesting a modulatory role by estrogens. Resko and his group recently demonstrated that estrogen administration significantly increases androgen receptor levels in rat anterior pituitary (Handa et al., 1987). Thus it is possible that estrogen priming was necessary in our system to induce adequate levels of androgen receptors to allow DHT to exert an effect. Alternatively, DHT could work through progesterone receptors, which require estrogen priming for their induction.

The effect of DHT on inhibiting estrogen-induced prolactin release was dependent on the dose used (Fig. 4): low doses (0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg) were inhibitory, whereas higher doses (3.2 mg/kg and 10 mg/kg) were much more variable in effect, and were not significantly different from the controls. The lack of effect of the higher doses of DHT is intriguing. High doses of DHT have been reported to bind estrogen receptors in vitro (Rochefort et al., 1979), whereas low or physiological concentrations of DHT seem to bind preferentially to androgen or progestin receptors (Jänne and Bardin, 1984). Dose dependency for progesterone's effect on gonadotropin release (McPherson and Mahesh, 1979), reduction of occupied nuclear estrogen receptors in the anterior pituitary (Fuentes et al., 1988), and on inhibition of estrogen-induced prolactin release (Brann, Putnam, and Mahesh, unpublished) has also been observed. The mechanisms involved in dose-dependent effects are unclear at this time.

The effect of DHT in inhibiting estrogen-induced prolactin release appeared to be mediated via the androgen receptor, since prior treatment with the antiandrogen, flutamide, blocked the DHT effect (Fig. 5). A surprising finding was the fact that flutamide also blocked progesterone's ability to inhibit estrogen-induced prolactin release (Fig. 7). One possible explanation could be that progesterone's action is mediated through the androgen receptor. Progesterone's ability to bind to the androgen receptor has been demonstrated by many investigators (Bullock et al., 1978; Wright et al., 1979; Jänne and Bardin, 1984; Sponda, 1984). In fact, progestin binding is now accepted as one of the features that distinguishes androgen receptor from extracellular androgen binding proteins such as ABP and TeBG (Jänne and Bardin, 1984). Additionally, the 5α-reduced metabolite of progesterone, dihydroprogesterone (DHP), is a highly effective competitor for the DHT receptor in rat ventral prostate (Wright et al., 1979), in the accessory sex glands of Syrian hamsters (Wright et al., 1978), and in human skin (Giacomini and Wright, 1980). Interestingly, DHP is a more effective competitor than testosterone for the rat prostate DHT receptor (Wright et al., 1979). Thus, flutamide's block of progesterone's action in this study could indicate that progesterone's action is mediated through the androgen receptor.

An alternative explanation could be that flutamide is exhibiting "antiprogestational" activity. In support of this is the recent report by Chandrasekhar and Armstrong (1988) that while flutamide does not bind to the progesterone receptor, it does significantly suppress serum progesterone levels and progesterone receptor levels in rat uteri. This "antiprogestational" action of flutamide offers an alternative explanation as to why flutamide blocked progesterone's action in our system.

RU486, a potent progesterone receptor antagonist, which competitively inhibits progestin binding (Bailieu, 1987), also blocked progesterone's ability to inhibit estrogen-induced prolactin release (Fig. 7). Since RU486 is a competitive inhibitor of progestin action, this evidence argues strongly that progesterone's action is mediated through its own receptor.

To establish more clearly that the DHT effect is an androgen receptor-mediated event and not progestin receptor-mediated, the competitive progesterone receptor antagonist, RU486, was used. As shown in Figure 7, RU486 pretreatment effectively blocked DHT's ability to attenuate estrogen-induced prolactin release. This could be interpreted as evidence of progestin receptor involvement in the mediation of DHT's action. Howe-

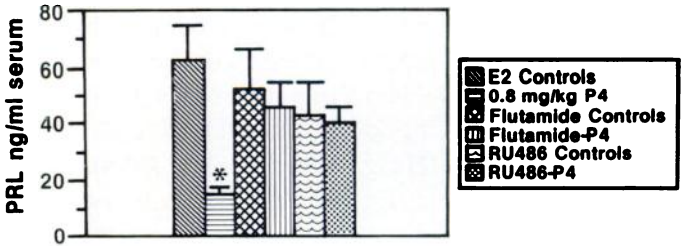

FIG. 7. Effect of progesterone upon estrogen-induced prolactin *(PRL)* release and the effect of the antiandrogen, *flutamide*, and the antiprogestin, *RU486*, upon the ability of progesterone to inhibit estrogen-induced PRL release. The protocol is the same as in *Figure 1*, and in *Figures 5* and *6* for the antagonist groups. *$p<0.01$.

ver, a review of the literature revealed that in addition to exhibiting high affinity for progestin and glucocorticoid receptors (Philibert et al., 1982), RU486 also binds to androgen receptors (with 25% the affinity of testosterone) and inhibits testosterone actions in a dose-dependent manner (Philibert, 1985). It is suggested that RU486's block of DHT's effect in our system could be via its ability to bind and antagonize the androgen receptor. Still, possible DHT interaction with progestin receptors cannot be ruled out. As stated earlier, progesterone and its 5α-reduced metabolite, DHP, are effective competitors for the DHT receptor in several tissues (Wright et al., 1978, 1979; Giacomini and Wright, 1980). Conversely, testosterone and its 5α-reduced metabolite, DHT, are effective competitors of progesterone binding in human, rabbit, and rat uteri (Haubbamaa and Luukkainen, 1975; Jänne and Bardin, 1984). The reciprocal affinities of these receptors for both androgens and progestins seem to indicate that each receptor system has a common binding site. Since both flutamide and RU486 were effective in blocking action by both DHT and progesterone, it is possible that DHT and progesterone action may be exerted in areas of receptor system that are also functionally common. This concept is in agreement with an observed overlap in biological effects of naturally occurring and synthetic androgens and progestational agents (Jänne and Bardin, 1984). Of particular interest was the finding that estrogen priming was needed for DHT action. Although estrogen priming may enhance androgen receptors, it appears to be essential for inducing progesterone receptors. More work remains to be done to clarify this possibility.

The precise mechanism by which DHT inhibits estrogen-induced prolactin release is not clear. We have demonstrated previously that progesterone's mechanism of inhibiting estrogen-induced prolactin release is via decreasing nuclear estradiol binding in the anterior pituitary (Calderon et al., 1987; Brann et al., 1988). A similar mechanism of action could be possible for DHT. Androgen suppression of estrogen receptor levels has been reported in immature rat ovaries (Saiduddin and Zassenhaus, 1978) and in rat pituitary and human breast cancer cells (Haug, 1979; Poulin, 1988). DHT has been reported to significantly decrease estradiol uptake and binding by lactotropes in ovariectomized rats (Keefer et al., 1987). Thus, DHT's effect, like progesterone's, could be due to suppression of estradiol binding in the anterior pituitary. An effect of DHT on opioid or dopaminergic systems, which are known to be important in regulating prolactin release, cannot be ruled out. These systems were not investigated in our study. It should be noted, though, that DHT has been reported to be unable to inhibit ether-induced prolactin release (Celotti et al., 1982), possibly indicating a specificity of its effects for estrogen-induced prolactin secretion.

The physiological significance of DHT inhibition of estrogen-induced prolactin release remains to be determined. However, several in vivo situations suggest a possible physiological role for 5α-DHT regulation of estrogen-induced prolactin release. First, at a time when 5α-DHT formation and levels are the highest in a female rat's life (Day 10–15) (Denef, 1983), prolactin serum levels are very low, even though estrogen levels are high (Dohler and Wuttke, 1974). Conversely, aging constant-estrous rats that display low androgen serum levels but normal estrogen serum levels, as compared to young cycling female rats, have significantly elevated basal prolactin serum levels (Lu et al., 1979). Lu et al. (1979) suggested that the elevated prolactin levels in constant-estrous rats are due to unopposed estrogen action in these animals.

Second, prolactin is an important component of the reproductive process. Some of its actions in vivo are regulation of growth and secretion of breasts (Meites and Shelesnyak, 1957), induction of luteinizing hormone receptors in ovaries (Richards and Williams, 1976), and maintenance of the corpus luteum during pregnancy in rats (Gibori et al., 1979). Most recently, prolactin has been shown to modulate immune response through newly discovered prolactin receptors on T lymphocytes (Russell et al., 1984). Thus, DHT antagonism of estrogen-induced prolactin release may be an important mechanism to regulate function of both the reproductive and the immune system in vivo.

Lastly, testosterone and 5α-DHT are regulators of gonadotropin secretion in males (Kalra and Kalra, 1983). In women, a hyperandrogenic state accompanied by alterations in gonadotropin secretion is well documented and described under the syndrome complex of polycystic ovaries (Mahesh, 1983; Mahesh et al., 1987). It has been presumed that the action of androgens in altering gonadotropin secretion is either direct or through their conversion to estrogens. The findings in this study indicate that the possibility of androgen interference with estrogen action also needs to be considered.

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