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BRIEF COMMUNICATION

Further Studies on the Restoration of Estrogen-Induced Sexual Receptivity in Ovariectomized Mice Treated with Dihydrotestosterone: Effects of Progesterone, Dihydroprogesterone and LH-RH[1]

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LUTTGE, W. G. AND C. S. SHEETS. *Further studies on the restoration of estrogen-induced sexual receptivity in ovariectomized mice treated with dihydrotestosterone: effects of progesterone, dihydroprogesterone and LH–RH.* PHARMAC. BIOCHEM. BEHAV. 7(6) 563–566, 1977. – Sexual receptivity in ovariectomized CD–1 mice induced by chronic daily injections of estradiol benzoate (E_2B) was inhibited in a dose related fashion by daily injections of dihydrotestosterone (DHT) given concurrently with the E_2B. Administration of the progestins, progesterone and dihydroprogesterone (DHP), and of the hypothalamic decapeptide, LH–RH, 6 hr prior to testing restored receptivity to varying degrees in these E_2B + DHT treated mice. Of these treatments progesterone was clearly the most effective, followed by LH–RH and finally DHP in restoring estrogen-induced receptivity. Results were discussed in terms of a proposed essential role for LH–RH in the induction of sexual receptivity in mice.

Sexual receptivity Estrogen Dihydrotestosterone Dihydroprogesterone Progesterone LH–RH

SEXUAL receptivity in ovariectomized mice and rats can readily be induced with either an acute series of one or two estradiol benzoate (E_2B) injections followed 24–48 hr later by a single progesterone injection 3–6 hr prior to testing, or by giving these females a chronic series of daily E_2B injections (e.g. [4, 6, 7, 14]). Furthermore, in rats and in the CD–1, but not the Swiss Webster, strain of mice the 5α-reduced metabolite of progesterone, dihydroprogesterone (DHP), can also effectively stimulate receptivity in ovariectomized E_2B-primed females [9, 12, 14, 20]. Since DHP synthesis and intracellular accumulation has been demonstrated in the brain it has been hypothesized that this metabolite may actually mediate some of the essential actions of progesterone in the brain [10, 12, 16, 20, 21]. (Steroid Trivial Names: dihydroprogesterone = 5α-pregnan-3,20-dione; dihydrotestosterone = 5α-androstan-17β-ol-3-one; estradiol benzoate = 1,3,5(10)-estratrien-3, 17β-diol 3-benzoate; progesterone = 4-pregnen-3,20-dione; testosterone = 4-androsten-17β-ol-3-one.)

As part of our continuing effort to develop pharmacological probes to study the mechanisms of ovarian hormone action in the brain, we recently conducted two series of experiments examining the interactions of the 5α-reduced metabolite of testosterone, dihydrotestosterone (DHT), with E_2B, DHP and progesterone in the induction of receptivity in mice [12,14]. We originally chose to examine the effects of DHT in female CD–1 and Swiss Webster mice, since our earlier work with long-term castrated males from these two out-bred albino strains indicated that DHT was effective in stimulating male sexual behavior only in the Swiss Webster strain [11,13]. Thus, the between strain pattern of behavioral efficacy was opposite for the 5α-reduced metabolite of progesterone in females from that of the 5α-reduced metabolite of testosterone in males. In our first study with DHT in female mice we found that giving an injection of this steroid, concurrent with the two daily E_2B injections and with the progestin injection given 6 hr prior to testing, inhibited the induction of receptivity in

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CD–1 ovariectomized females when the progestin was DHP, but not when the progestin was progesterone [12]. From this study we concluded that DHT had specifically inhibited the behavioral actions of DHP, while sparing those of progesterone. In our second study with DHT in female mice we found that daily injections of this steroid inhibited the display of receptivity in ovariectomized CD–1 females receiving chronic daily injections of E_2B [14]. We also found that a single injection of progesterone given 6 hr prior to testing partially restored receptivity in these DHT-treated females. Similar injections of DHP had no restorative effects on receptivity. From this and other studies we concluded that DHT was having an anti-estrogenic effect and that progesterone, but not DHP was able to overcome those effects as indicated by the restoration of estrogen-induced sexual receptivity. However, in this work only a single dose of DHT (1 mg/mouse/day) and of the progestins DHP and progesterone (500 μg/mouse) were investigated. Thus, if lower doses of DHT and/or higher doses of the progestins were investigated, it is entirely possible that progesterone could be found to completely restore receptivity to pre-DHT levels, and/or that DHP could also be found to have restorative effects on DHT-inhibited receptivity.

In the present study we examined these dose-response relationships for DHT, DHP and progesterone. We have also tested one possible mechanism of DHT action in the inhibition of estrogen-induced receptivity, namely, that the daily injections of DHT were blocking behaviorally essential actions of the endogenous hypothalamic decapeptide, luteinizing hormone releasing hormone (LH–RH). This hypothesized LH–RH mechanism for DHT action seemed plausible for four reasons. First, in previous work we found that DHT did not inhibit ^3H-estradiol accumulation in nuclear fractions obtained from hypothalamic and preoptic brain regions [14]. Thus, DHT appears to either block postbinding actions of estrogen which are essential for the induction of receptivity or else block receptivity by a mechanism independent of the direct estrogen-receptor interactions in the brain. Second, since progesterone has been shown to be very effective in stimulating the release of LH and LH–RH in estrogen-primed rats (e.g. [1, 2, 16, 21]), it is possible that the restorative actions of progesterone on DHT-inhibited receptivity could also be mediated by its actions on endogenous LH–RH. Third, systemic and intracerebral treatment with LH–RH has been shown to facilitate the induction of receptivity in estrogen-primed female rats (e.g. [8,17]), and in pilot work in our laboratory, we found a similar facilitation in estrogen-primed CD–1 female mice (Luttge and Hall, unpublished observations). Fourth, hypophysectomy has also been shown to facilitate the induction of receptivity with estrogen priming in ovariectomized rats [3]. Since this procedure has also been shown to elevate endogenous LH–RH plasma levels [15,18], and since DHT has potent negative feedback actions on the secretion of LH and on the action of LH–RH in the pituitary (e.g. [5, 13, 19]) as well as inhibiting the hypophysectomy-induced facilitation of receptivity in rats [3], it seemed likely that the inhibitory actions of DHT on sexual receptivity were in some way related to the actions of LH–RH. Thus, if DHT inhibits the behaviorally essential actions, synthesis or release of LH–RH in the brain, then treatment with additional exogenous LH–RH should be successful in restoring estrogen-induced receptivity in DHT-treated mice.

METHOD

Twenty-one adult CD–1 female mice (Mean body wt = 30.05 g), purchased from Charles Rivers Laboratories (Wilmington, MA), were housed individually with food and water available at all times. The animal colony lights were left on from 2100 to 0900 hr and all injections and testing were performed during the first eight hr of the dark phase of the lighting cycle. All females were ovariectomized under methoxyflurane anesthesia and started on a daily SC injection schedule of 2 μg E_2B/mouse. After 10–11 days of estrogen priming all females were tested for sexual receptivity as described below and the 18 females displaying the highest L/M scores were placed into one of three groups of six and given SC injections of 100, 200 or 400 μg DHT/mouse/day concurrent with their daily E_2B injections. All females were then retested for sexual receptivity after 5, 6, 10, 11, 15, 16, 20 and 21 days of this injection schedule (all tests approximately 24–26 hr after the last E_2B + DHT injection). Six hr prior to testing on Days 11, 16 and 21 of E_2B + DHT treatment all three groups of females were given SC injections of 1000 μg DHP, 500 μg progesterone or 500 ng LH–RH, respectively. After the test on Day 21 of E_2B + DHT injections the DHT treatment was halted and the females were tested for sexual receptivity after five additional days of E_2B treatment. The injection and testing schedule used in this experiment is illustrated in Fig. 1.

The E_2B, progesterone, DHP and DHT used in this study were purchased from Steraloids, Inc. (Wilton, NH), while the LH–RH (AY–24,031) was generously provided by Ayerst Research Laboratories (Montreal, Canada). All steroids were dissolved in a benzyl benzoate-peanut oil solution (20:80, v:v) in different concentrations such that all injections, including the combined E_2B + DHT injections, were given in 0.1 cc volumes. The LH–RH was dissolved in saline and injected in 0.2 cc volumes.

All sexual behavior testing was done in the animal colony under dim red light illumination and without the observer's knowledge of the group from which each individual animal was derived. Each test was initiated by placing the test female in a 12.5 × 28 × 28 cm circular glass testing arena containing a sexually vigorous CD–1 male mouse. Males were permitted to mount 10 times and the quality of the females' response to each of these mounts recorded. Only mounts with pelvic thrusting were counted. If a given male failed to either initiate or continue mounting, the female was moved to another testing arena containing a different male. All responses to mounts were scored on a four point scale with a score of zero indicating a negative response with the female's back remaining convexly curved and the head pointed down. With a score of one the female's perineal region had to be raised, but the head remained down, whereas with a score of two the perineal region and the head were both raised with the female's body level with respect to the ground. Finally, with a score of three the perineal region and head had to be raised to such an extent that the female's back was in a pronounced concave arch. Females exhibiting posture scores of two or three typically permitted the male to achieve intromissions. These two top scores were also defined as lordoses and used to compute the lordosis quotient measure of receptivity

$$(L/M = \frac{\text{No. Lordoses}}{\text{No. Mounts}} \times 100).$$

Since graphs and statistical analyses of the median quality scores for each group did not differ from similar graphs and statistical analyses of the mean L/M scores, only the latter measures have been included in this report.

RESULTS

The L/M scores observed during each of the tests are illustrated in Fig. 1. All three doses of DHT were found to significantly reduce the L/M scores after five, and especially after 10 days of combined E_2B + DHT treatment ($p<0.01$, F tests). The 100 μg/day DHT treatment paradigm was found to be slightly less effective than the 200 and 400 μg/day treatment paradigms which failed to differ in apparent potency. The 1000 μg DHP treatment given 6 hr prior to testing on Day 11 of the E_2B + DHT injections produced slight, but non-significant ($p>0.05$) increases in the L/M scores in the 200 and 400 μg/day DHT treatment groups. In the 100 μg/day DHT treatment group the DHP treatment more than doubled the L/M scores compared to the test on the previous day without DHP ($p<0.05$, paired t-test). However, even with this dramatic increase in the L/M score in the 100 μg/day DHT treatment group the levels of receptivity displayed after DHP treatment were still significantly lower than those displayed before initiation of the combined E_2B + DHT injections ($p<0.05$, paired t-test). The L/M scores declined after an additional four days of E_2B + DHT injections such that they were now equivalent to those displayed before the DHP injections. The 500 μg progesterone treatment given 6 hr prior to testing on the next day produced a dramatic, clearly significant ($p<0.01$, paired t-tests) increase in L/M scores in all three DHT treatment groups. The levels of receptivity displayed in this test were equivalent to those displayed prior to the initiation of DHT treatment. After four additional days of combined E_2B + DHT injections the L/M scores had once again declined in a DHT dose related fashion to levels equivalent to those displayed prior to progestin treatment. The treatment with 500 ng LH–RH 6 hr prior to testing on Day 21 of combined E_2B + DHT injections also increased the levels of receptivity displayed in all three treatment groups, but this facilitation was only significant in the 100 and 400 μg/day DHT treatment groups ($p<0.05$, paired t-tests). The levels of receptivity displayed after LH–RH treatment were still less than those displayed prior to the start, and five days after the cessation, of the DHT treatment ($p<0.05$, paired t-tests). The L/M scores displayed during this last test after the cessation of the DHT treatment were equivalent to those displayed prior to the start of the combined E_2B + DHT injections.

DISCUSSION

The present results have replicated and expanded upon our earlier demonstrations [12,14] that concurrent administration of DHT with daily injections of E_2B can effectively inhibit the display of sexual receptivity in ovariectomized CD–1 mice. An examination of the three DHT doses used in this experiment indicates that while the 200 and 400 μg/day DHT treatments produced near maximal effects, roughly equivalent to our earlier results with a 1000 μg/day DHT treatment schedule [14], the 100 μg/day DHT treatment schedule produced a partial, but still significant inhibition of E_2B-induced sexual receptivity. The reduced DHT doses used in the present study com-

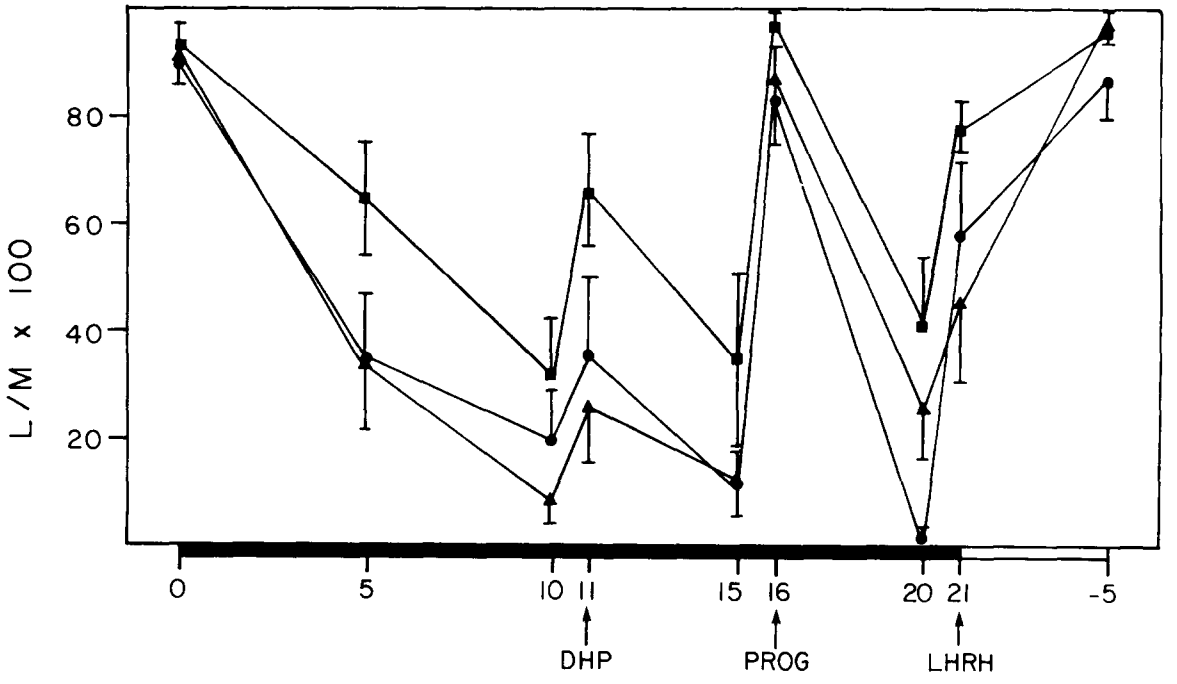

FIG. 1. Lordosis quotients ($\bar{X}$ ± SEM) for E_2B-primed ovariectomized mice. Solid bar along the abscissa indicates period during which females received 100 (squares), 200 (triangles) or 400 (circles) μg DHT/day concurrent with their daily 2 μg E_2B injections. The open bar indicates the five day period during which all females received only the E_2B treatment. The arrows beneath Days 11, 16 and 21 of E_2B + DHT treatment indicate additional injections of 1000 μg DHP, 500 μg progesterone or 500 ng LH–RH 6 hr prior to testing, respectively.

bined with a doubling of the DHP dose compared with our earlier studies, has further revealed that in contrast to our earlier studies DHP can also at least partially restore DHT-inhibited receptivity in a similar fashion to that demonstrated earlier for progesterone [14]. However, progesterone is still clearly more effective than DHP in restoring estrogen-induced receptivity since injections of half as much progesterone as DHP (i.e., 500 vs. 1000 μg/mouse) produced a dramatic increase in sexual receptivity in all three E_2B + DHT treatment groups. Further comparisons of these results with our earlier work with the 1000 μg/day DHT treatment schedule [14] suggests that the degree of progesterone-induced restoration of sexual receptivity can be reduced with higher doses of DHT.

The present results have also demonstrated that SC administration of the hypothalamic decapeptide LH–RH can at least partially restore estrogen-induced receptivity in DHT-treated ovariectomized mice. These findings clearly support our pilot observations that LH–RH can facilitate the induction of receptivity in estrogen-primed ovariectomized mice pretreated with dexamethasone to suppress adrenal progesterone (Luttge and Hall, unpublished observations). In this earlier pilot study receptivity was significantly increased compared to pretreatment scores and saline-treated controls within 2½ hr after LH–RH injection. Since the L/M scores continued to increase in tests performed out to 6½ hr after LH–RH administration, we initiated our testing in the present study 6 hr after the SC injections of LH–RH. In the Introduction to this report we outlined four reasons why it seemed plausible to investigate the possible involvement of LH–RH in the mechanisms of DHT-inhibition of estrogen-induced receptivity. The success of exogenous LH–RH administration in restoring receptivity suggests that the daily administration of DHT may block the synthesis, release or action of endogenous LH–RH which is essential for the mediation of estrogen-induced receptivity. Future experiments will be required to resolve these various possibilities. The fact that progesterone is clearly more potent than DHP in restoring receptivity in DHT-treated mice ([12,14], Fig. 1) that progesterone has been shown to be much more potent than DHP in stimulating the release of LH in estrogen-primed ovariectomized rats [2, 14, 21], further suggests that the mechanism of progestin-induced restoration of sexual receptivity in E_2B + DHT treated mice also involves the actions of endogenous LH–RH. Direct tests of this hypothesized mechanism must await future experimentation.

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