Vol. 86, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1979 Pages 1214-1221

INTERFERENCE OF GONADOTROPIN-RECEPTOR INTERACTION BY SYNTHETIC ESTROGENS

M.R. Sairam and M.I. Berman

Reproduction Research Laboratory
Clinical Research Institute of Montreal
110 Pine Avenue West
Montreal, Quebec H2W 1R7, Canada

and the University of Montreal

Received January 19, 1979

SUMMARY

In *in vitro* studies, the synthetic estrogens diethylstilbestrol and diethylstilbestrol sodium phosphate inhibited the binding of ^{125}I ovine lutropin to the rat ovarian receptor and ^{125}I ovine follitropin to the bovine testicular receptor. As the lutropin binding to receptor is affected to a greater extent, a preferential inhibitory effect may be suggested. Removal of the estrogens from the incubation medium by washing does not restore gonadotropin binding ability, indicating a strong effect. The two compounds were effective in displacing the labeled gonadotropin from the preformed receptor-hormone complex. This effect increased with time of incubation. It appears unlikely that the interference of gonadotropin-receptor interaction may be because of increased hormone and/or receptor degradation by the two compounds.

INTRODUCTION

Like many other hormones a principal site of action of the gonadotropins appears to be at the level of the cell membrane. Accordingly, specific receptors for lutropin (LH) and follitropin (FSH) have been demonstrated in the ovary and testis of different animals (see 1,2). Very few studies have been carried out regarding the influence of the end products such as the steroids on the hormone binding itself. During a recent study on the direct effects of estrogenic compounds on lutropin action on rat Leydig cells (3) we obtained evidence to suspect that receptor-hormone interaction might be affected. We have now extended these studies and demonstrate here that synthetic estrogens interfere with gonadotropin-receptor interaction in both the ovary and the testis.

Abbreviations used: LH--lutropin; FSH--Follitropin; HCG--Human Chorionic Gonadotropin; E2--Estradiol 17β; E2B--Estradiol benzoate; P--Progesterone; T--Testosterone; A--Androstenedione; DES--Diethylstilbestrol; DES-NaP--Diethylstilbestrol sodium phosphate (Honvol).

0006-291X/79/041214-08$01.00/0

Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

A crude membrane fraction from pseudopregnant rat ovaries (4) was used as a preparation of highly specific lutropin receptor. Follitropin receptor in the form of a partially purified membrane fraction was prepared from adult bull testis (5) using a modified procedure (6). Both receptor preparations were employed either immediately or after being frozen at -70° for several months, as no difference in binding ability was perceptible between the two forms. Highly purified ovine pituitary lutropin and follitropin isolated in this laboratory and human follitropin supplied by NIH were used for iodination with carrier free ^{125}I (6) by the lactoperoxidase method. The labeled hormones purified on Sephadex G-100 column had a specific activity of about 50-80 μCi/μg and exhibited good binding characteristics. The labeled hormones were used within two weeks of preparation.

Binding assays were done in disposable 12 x 75 mm glass tubes. Each incubation mixture contained 100 μl of the sample in the assay buffer and 400 μl of a suspension of the appropriate receptor plus the labeled hormone added as a single aliquot. The total volume was 0.5 ml in the assay buffer, which was 25 mM Tris-HCl, pH 7.5 containing 10 mM $MgCl_2$ and 0.1% bovine serum albumin (Sigma). The tubes were incubated at 37°C for two hours at the end of which the reaction was terminated by addition of 2 ml cold assay buffer. The tubes were centrifuged at about 2900 x g for 10-15 mins. After aspiration of the supernatant, the bound radioactivity in the pellet was counted in a Beckman autogamma Spectrometer. Tubes designated for non-specific binding contained 1000 fold excess of the unlabeled hormone.

The compounds to be evaluated for effects on hormone-receptor interaction, were dissolved in ethanol. Suitable controls receiving the same concentration of ethanol were always employed.

In experiments where the effect on preformed hormone-receptor complex was examined, the receptor preparation was incubated as above with the labeled hormone at 37°C for 2 hrs and washed once with buffer and then resuspended in the original volume of 0.5 ml containing either buffer, ethanol, hormone or the various compounds. They were incubated at 37°C and at specified intervals (up to 24 hrs) triplicate sets were removed, diluted with buffer and the pellet separated again as above. Radioactivity in the pellet was determined and expressed as percentage of the original binding found during the formation of the first 2 hr receptor-hormone complex.

The steroids E2, E2B, P, T and A and the DES were obtained from Sigma, St.Louis. The clinically used form of DES as its sodium phosphate derivative was donated by Horner Laboratories, Montreal. This compound supplied as an aqueous solution was directly diluted with the assay buffer. DES-NaP concentrations are shown in DES equivalents.

All solutions were prepared fresh just prior to use.

RESULTS

Effects on binding of ^{125}I lutropin and ^{125}I follitropin to their receptors

Among the different estrogenic compounds and other steroids tested, only the synthetic estrogens DES and DES-NaP significantly interfered with binding of the labeled gonadotropins to the receptors as seen in Fig. 1.

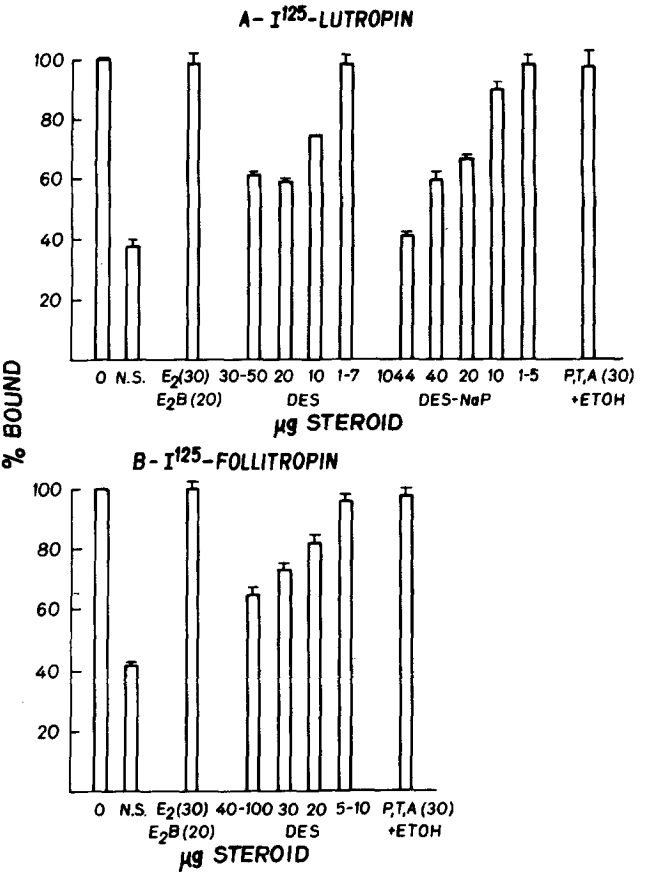

Figure 1. Effect of estrogens on ^{125}I-lutropin (A) and ^{125}I human follitropin (B) binding to receptor. In each experiment about 1 mg ovarian or testicular membrane protein was incubated with 80-100,000 cpm of labeled hormone (about 1 ng) and the various compounds. The tubes with a total volume of 0.5 ml (see methods) were incubated at 37°C for 2 hrs. The % bound in absence of the unlabeled hormone (zero binding) which was 20% of the added radioactivity is considered as 100%. Non specific binding in this and other experiments represent cpm found in presence of 1 µg of highly purified unlabeled hormone. Data in this and subsequent figures represent mean ± SEM. In subsequent studies E2, E2B, P, T, A were found to be without influence at 50-100 µg. EtOH = ethanol control. The DES-NaP also gave a dose dependent inhibition (20-400 µg) in panel B (data not shown).

DES at approximately 0.037 mM reduced the binding of ^{125}I lutropin to the ovarian receptor by about 50%. The maximum inhibition of specific binding seen at higher concentrations was of the order of 75-80% in 2 hrs. It thus appears that the degree of inhibition is dose related (Fig. 1A). Very similar effects were observed with DES-NaP. The inhibitory effect

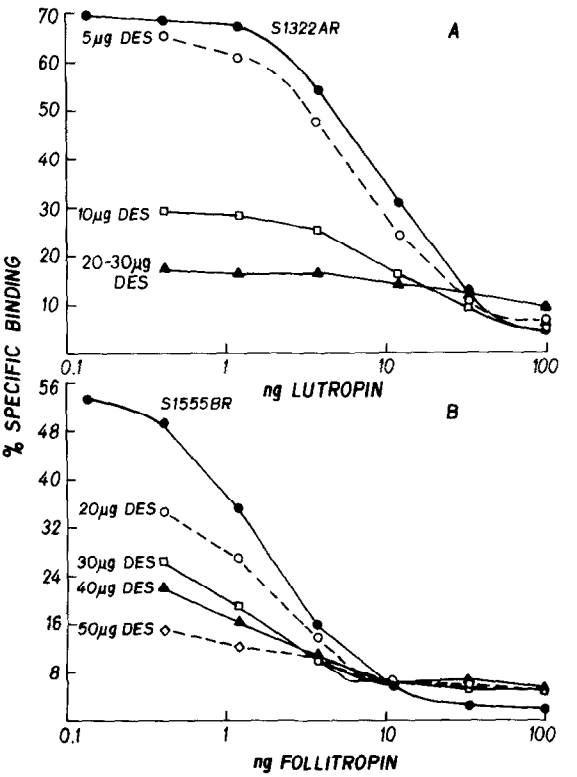

Figure 2. Influence of varying DES levels on the radioreceptor assay dose-response of A-lutropin and B-follitropin (human). S1322AR and S1555BR represent highly purified ovine lutropin and follitropin isolated in this laboratory.

of DES on follitropin-receptor interactions (Fig. 1B) was much less than that observed with lutropin.

The dose dependent effect of DES on the two systems is more clearly evident in Fig. 2A and 2B, where the radioreceptor assay responses were evaluated in presence of increasing concentrations of DES. In presence of 20 μg DES, the lutropin dose-response is virtually obliterated, whereas with follitropin and its receptor, the slope is reduced progressively.

Effects of preincubation with estrogens and washing on gonadotropin binding

In these experiments, the respective receptor preparations were first incubated with the two compounds for 2 hrs at 37°C. The membrane pellets, recovered by centrifugation were washed thrice with the assay

buffer and then reincubated in presence of the ^{125}I-lutropin or ^{125}I-follitropin for another 2 hrs. The inhibition of subsequent gonadotropin binding, caused by preincubation of the lutropin receptor with 20 µg DES or DES-NaP, or the follitropin receptor with 50 µg of the same compounds, could not be reversed by the washing procedure. Since DES-NaP is easily soluble in aqueous buffers, the loosely bound compound would be effectively removed by washing. These results suggest an effective alteration of gonadotropin binding sites on the membranes.

Effects on preformed gonadotropin-receptor complex

Both DES and DES-NaP enhanced the dissociation of the preformed gonadotropin-receptor complexes as shown by the experiments described in figure 3. With the ovarian lutropin receptor-hormone complex, incubation at 37°C over 24 hrs led to the loss of the bound hormone in absence of any additives (Fig. 3A). This release was increased by the presence of the unlabeled lutropin. Inclusion of the synthetic estrogens also enhanced the rate of loss of the bound hormone and at the end of 24 hrs, only a fraction of the labeled lutropin remained bound to its receptor. In contrast to lutropin receptor, the bull testis follitropin complex was relatively more stable (Fig. 3B). Although significant dissociation of the bound hormone occurs, a substantial amount still remains bound after 24 hrs. As in Fig. 3A, bound hormone was displaced by unlabeled follitropin, or the synthetic estrogens. It may be noted that, again higher concentration of the estrogens were necessary in the case of the follitropin receptor.

DISCUSSION

These studies demonstrate that structurally unrelated hormones, such as the synthetic estrogens can directly affect the specific interaction of lutropin and follitropin with the ovarian and testicular receptors respectively. As indicated elsewhere (3) the estrogenic compounds also effectively interfere with lutropin binding to the rat testicular receptor. It may be noted that

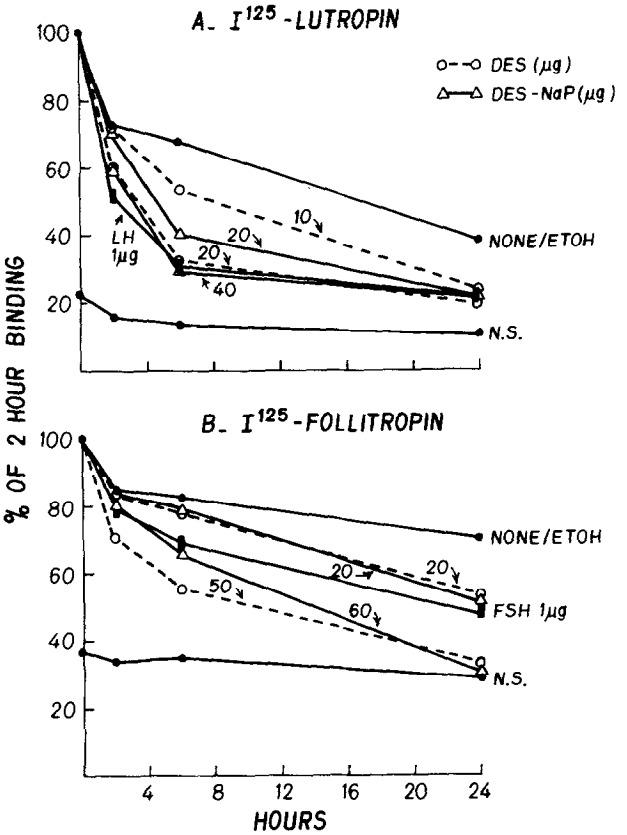

Figure 3. Effect of the synthetic estrogens on the preformed hormone-receptor complex. A-lutropin, B-ovine follitropin. Incubations were done as shown in figure 1. After the formation of the initial gonadotropin-receptor complex (2 hrs), the pellet was washed once with the assay buffer and reincubated at 37°C in the original volume (0.5 ml) with the indicated substances. At specified intervals triplicates were removed and the reaction stopped by addition of 2 ml cold buffer, centrifuged and radioactivity in the pellet determined. All values are represented as % of the original binding found during the first 2 hr incubation which was 40% and 42% of the added CPM in A and B respectively. For reasons of clarity the SEM bars are omitted as triplicates varied less than 3%. EtOH = ethanol. NS = non specific binding.

the concentrations required for the inhibition are rather high. For instance, the ID50 for inhibition of ^{125}I lutropin binding to the ovarian receptor is 0.037 mM and 1 nM respectively for DES and ovine lutropin (Figs. 1 and 2). It has been noted recently that in hypophysectomized rats, DES at lower concentrations, can significantly reduce ^{125}I-hCG binding to the testicular receptors (7) indicating the greater sensitivity of the hormone-deprived tissue.

In all the experiments shown here, the binding of follitropin to the testicular receptor was affected to a much lower degree at equivalent concentrations (Figs. 1, 2 and 3). However, it may be noted that preincubation of the receptor with a large dose of DES, such as 50 µg prevented subsequent follitropin binding. In the hypophysectomized male rat, DES treatment *in vivo* preferentially affects lutropin receptor without significantly altering follitropin binding (7).

The mitogenic action of the estrogen DES, has frequently been utilized to increase the number of granulosa cells and thus the number of follitropin binding sites in the ovary (see 8,9). Such cells apparently cannot bind labeled hCG (cited in 10) and fail to produce progesterone in the presence of this hormone (11). The direct preferential effect of the DES and DES-NaP on lutropin receptor *in vitro* noted in the present studies might explain some of these *in vivo* observations.

The mechanism of action of the synthetic estrogens remains unknown. It is possible that the DES or DES-NaP may bind to membrane (receptor) components and subsequently induce conformational changes resulting in decreased binding ability of the gonadotropin receptors. This supposition is supported by the data which showed that the synthetic estrogens were effective in dislodging the labeled hormone from the preformed hormone-receptor complex (Fig. 3). Preincubation of the membrane preparations with the estrogens followed by washing effectively blocked subsequent gonadotropin binding. We are not aware of studies which have shown a direct binding of labeled DES or DES-NaP to the gonadotropin receptor or peptide, protein hormone receptors. It is unlikely that the estrogens could be enhancing the degradation of the hormone and/or the receptor.

ACKNOWLEDGMENTS

This investigation was supported by grants from the MRC of Canada. DES-NaP (Honvol) was generously supplied by Horner Laboratories, Montreal. The human FSH (NIH-HS-1) was supplied by the Hormone Distribution Officer at NIAMDD, Bethesda.

REFERENCES

1. Ryan, R.J. and Lee, C.Y. (1976) Biol. Reprod. 14, 16-29.

2. Catt, K.J. and Dufau, M.L. (1976) Biol. Reprod. 14, 1-15.

3. Sairam, M.R. and Berman, M.I. Steroids (Submitted for publication).

4. Lee, C.Y. and Ryan, R.J. (1975) J. Clin. Endocrinol. Metab. 40, 228-233.

5. Cheng, K.W. (1975) J. Clin. Endocrinol. Metab. 41, 581-589.

6. Sairam, M.R. J. Endocrinol., in press.

7. Hsueh, A.J.W., Dufau, M.L., and Catt, K.J. (1978) Endocrinology 103, 1096-1102.

8. Goldenberg, R.K., Vaitukaitis, J.L., and Ross, G.T. (1972). Endocrinology 90, 1492-1498.

9. Louvet, J.P. and Vaitukaitis, J.L. (1976) Endocrinology 99: 758-764.

10. Hillier, S.G., Knazek, R.A., and Ross, G.T. (1977) Endocrinology 100, 1539-1549.

11. Lucky, A.W., Schreiber, J.R., Hillier, S.G., Schulman, J.D., and Ross, G.T. (1977) Endocrinology 100, 128-133.