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Novel Presence of Luteinizing Hormone/Chorionic Gonadotropin Receptors in Human Adrenal Glands

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Abstract

It has been well documented that a significant proportion of chronic anovulatory patients have elevated levels of dehydroepiandrosterone sulfate (DHEAS) and luteinizing hormone (LH) and normal levels of adrenocorticotropic hormone (ACTH). We tested the hypothesis that the zones of human adrenal cortex that secrete DHEAS may contain LH/human chorionic gonadotropin (hCG) receptors. In situ hybridization showed the presence of hybridization signals representing LH/hCG receptor mRNA transcripts in the zona reticularis. Immunocytochemistry demonstrated that the zona reticularis also contained LH/hCG receptor protein. The receptor transcripts and receptor protein are also present in the deeper layer of the zona fasciculata which can also secrete DHEAS. Double immunostaining revealed that LH/hCG receptors are present in the same cells that contain cytochrome P450 side chain cleavage enzyme, suggesting that the receptor containing cells are steroidogenic. These findings may potentially explain higher DHEAS levels in chronic anovulatory women who have normal ACTH and elevated LH levels.

Introduction

Human adrenal glands contain structurally and functionally distinct layers of cortex and medulla (1). The cortex contains morphologically and functionally distinct zona glomerulosa which secretes mineralocorticoids, zona fasciculata which secretes glucocorticoids and androgens and zona reticularis which secretes androgens (1). Angiotensin II regulates the steroidogenesis in the zona glomerulosa and adrenocorticotropic hormone (ACTH) regulates the steroidogenesis in the zona fasciculata and reticularis (1). Dehydroepiandrosterone sulfate (DHEAS) is a weak androgen primarily secreted by the adrenals (2,3). The changes in DHEAS levels are not always accompanied by similar changes in ACTH (4-7). Therefore, it was suggested that other hormones may regulate the secretion of DHEAS (8,9). A significant proportion of chronic anovulatory patients have elevated levels of DHEAS and LH and normal levels of ACTH (6,10). This finding raises a suspicion that LH may stimulate adrenals to secrete higher levels of DHEAS. In fact, hCG, a structural and functional homolog of LH, can stimulate the secretion of androgens in cells isolated from guinea pig adrenals which are considered very similar to human adrenals in morphology and function (11). Moreover, hCG is a known stimulator of DHEAS secretion by human fetal adrenal glands during early pregnancy (12). These two findings suggest that adrenal zones that secrete DHEAS may possibly contain LH/hCG receptors. The possibility that human adrenals may have the LH/hCG receptors was strengthened by several recent studies which demonstrated that many human nongonadal tissues also contain functional LH/hCG receptors (13-25). In the present study, we investigated adult human adrenals for LH/hCG receptors by in situ hybridization and immunocytochemistry.

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Materials and Methods

Paraffin embedded blocks of four male and eight female human adrenal glands were obtained from the Pathology departments of hospitals affiliated with the University of Louisville School of Medicine.

In situ hybridization was performed using ^{35}S labeled riboprobes transcribed from a full length porcine LH/hCG receptor cDNA obtained from Dr. H. Loosfelt at Hopital Bicetre, France. Hybridization with ^{35}S labeled sense riboprobe served as a control. Additional details regarding the procedure have been previously described (15).

Immunocytochemistry was performed by an avidin immunoperoxidase method using a 1:350 dilution of polyclonal LH/hCG receptor antibody raised against a synthetic N-terminus amino acid sequence of 15-38 (15). The antibody and the corresponding synthetic receptor peptide were obtained from Dr. Patrick Roche from the Mayo Clinic, Rochester, MN. For the procedural controls, either the receptor antibody was preabsorbed with excess receptor peptide or unabsorbed receptor antibody was omitted or replaced with nonspecific IgG.

Double immunostaining was performed using 1:500 and 1:300 dilutions of antibodies to cytochrome P450 side chain cleavage enzyme and LH/hCG receptors, respectively. The polyclonal antibody raised against the human cytochrome P450 side chain cleavage enzyme was obtained from Dr. Walter Miller from the University of California, San Francisco, CA. The sections were first immunostained for cytochrome P450 side chain cleavage enzyme using 5-bromo-4-chloro-3-indolyl phosphate/4-nitro blue tetrazolium as the substrate for the enzyme, which gave a blue color. Then the

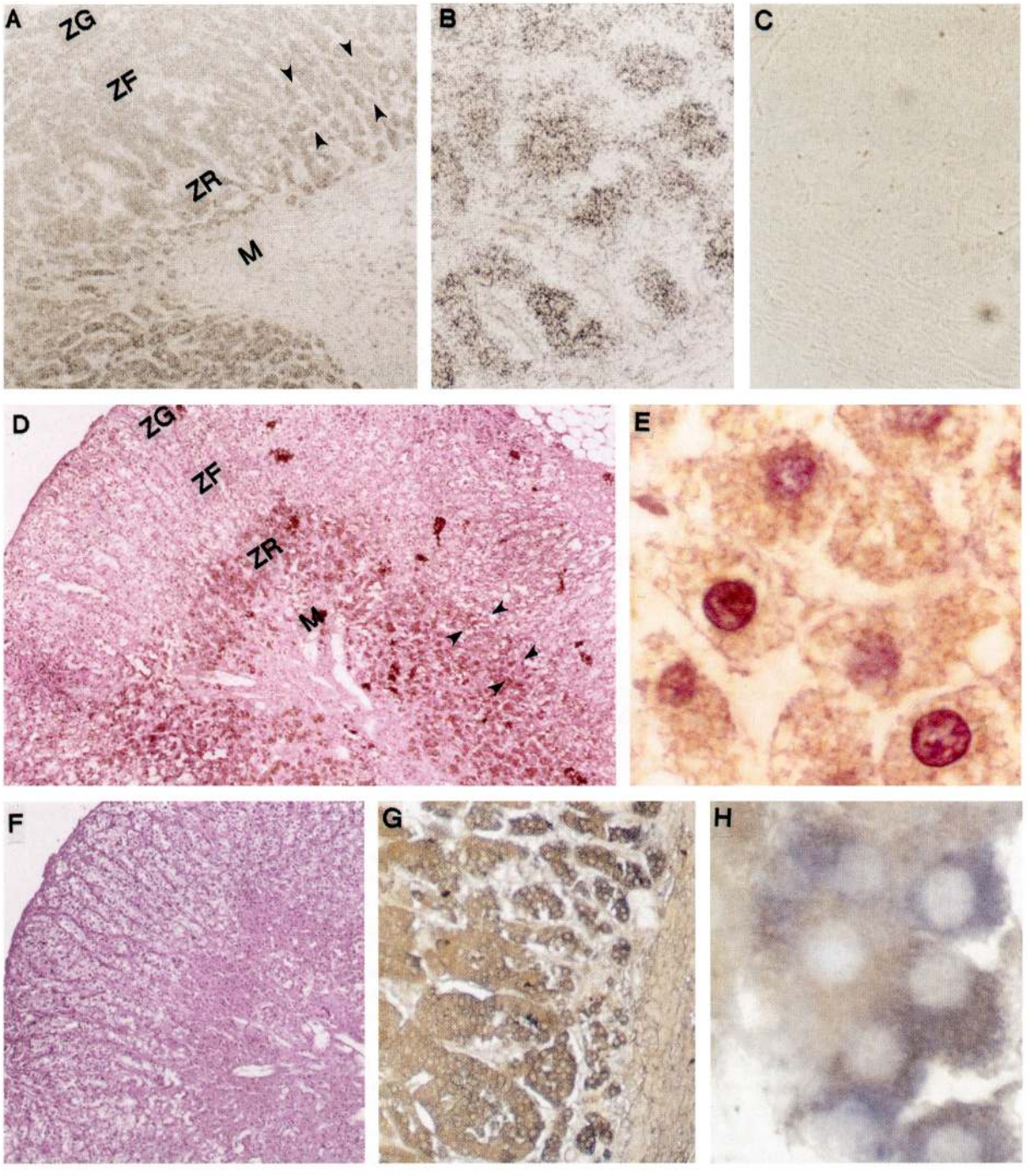

Fig. 1. In situ hybridization (A to C) and immunocytochemistry (D to F) for LH/hCG receptors and double immunostaining for LH/hCG receptors and cytochrome P450 side chain cleavage enzyme (G & H) in human adrenals. C is a sense in situ hybridization control and F is a preabsorption immunostaining control. The space between arrowheads in A and D represents the approximate area of the deeper layer of zona fasciculata. In G & H, the blue color represents cytochrome P450 side chain cleavage enzyme and the brown color represents LH/hCG receptors. The following abbreviations are used: ZG=zona glomerulosa; ZF=zona fasciculata; ZR=zona reticularis; M=adrenal medulla. Mag=A, D & F=150 X; B & C=600 X; G=300 X; E & H=1,500 X

sections were immunostained for LH/hCG receptors using diaminobenzidine as the substrate for the enzyme, which gave a brown color. The rest of the details are the same as previously described (23).

Results

We first performed in situ hybridization for LH/hCG receptor mRNA transcripts in human adrenal sections. As shown in Fig. 1A, the deeper layer of the zona fasciculata and the entire zona reticularis contained hybridization signals when ^{35}S antisense riboprobe transcribed from a full length porcine LH/hCG receptor cDNA was used. The zona glomerulosa and the adrenal medulla, on the other hand, contained very few or no hybridization signals. A higher magnification picture demonstrates that the cells in the zona reticularis, but not intercellular connective tissue, contain hybridization signals (Fig. 1B). In situ hybridization with ^{35}S sense riboprobe used for a control showed no signals (Fig. 1C). A nonradioactive method of in situ hybridization using a fluorescein II-UTP labeled 210 base antisense or sense riboprobes transcribed from human LH/hCG receptor cDNA also gave similar results (data not shown).

We next performed immunocytochemistry for LH/hCG receptor protein. As shown in Fig. 1D, immunostaining is present in the deeper layer of zona fasciculata and the entire zona reticularis, but not in the zona glomerulosa or the medulla. A higher magnification picture demonstrates that the cells, but not intercellular connective tissue, contain receptor immunostaining (Fig. 1E). The receptor immunostaining was absent when the receptor antibody was preabsorbed with excess receptor peptide (Fig. 1F) or when the unabsorbed receptor antibody was omitted or replaced with nonspecific IgG during the immunostaining procedure (data not shown).

We finally performed double immunostaining for cytochrome P450 side chain cleavage enzyme and LH/hCG receptors to determine whether the receptors are present in the steroidogenic cells. Figs. 1G and H show that the blue color, representing cytochrome P450 side chain cleavage enzyme, is present in the same cells that contain brown color, representing LH/hCG receptors.

Discussion

The present study was mainly prompted by the clinical paradox seen in a significant proportion of chronic anovulatory women who have elevated levels of DHEAS and LH and normal levels of ACTH (6,10). If the zona reticularis of adrenals was to contain LH/hCG receptors, then elevated LH levels could stimulate adrenals to secrete greater amounts of DHEAS. The possibility that the adrenal cortex may contain LH/hCG receptors was strengthened by the findings that hCG alone or in combination with low levels of ACTH, could stimulate the secretion of androgens by isolated guinea pig adrenal cells (11). In addition, it is well known that hCG can stimulate the secretion of DHEAS in human fetal adrenals during early pregnancy (12). The hCG effects, either in fetal or adult adrenals, suggest that LH/hCG receptors might be present, but to our knowledge, no one has ever investigated adrenals for the presence of LH/hCG receptors.

Due to the lack of availability, we could not investigate fresh adult human adrenal tissue for the presence of LH/hCG receptors. Therefore, we had to use paraffin embedded tissue blocks of adult human adrenal glands. The blocks came from men and women who died of causes that were unrelated to any adrenal disease. The results of in situ hybridization and immunocytochemistry experiments revealed that zona reticularis and the deeper layer of the zona fasciculata contain LH/hCG receptor transcripts and protein. The use of porcine and human probes gave similar in situ hybridization results. This receptor distribution is specific as the zona glomerulosa, the upper layer of zona fasciculata or the adrenal medulla did not contain detectable LH/hCG receptor transcripts or receptor protein. Moreover, the procedural controls demonstrated the absence of hybridization signals or immunostaining in the zona reticularis and the deeper layer of the zona fasciculata. Double immunostaining confirmed that the LH/hCG receptor containing cells are steroidogenic. Because of the small sample size, we cannot draw any conclusions concerning sex differences in adrenal LH/hCG receptors.

We were unable to investigate adrenals of chronic anovulatory women for LH/hCG receptors. Nevertheless, the presence of LH/hCG receptors in the zona reticularis and the deeper layer of the of the zona fasciculata may possibly explain higher levels of DHEAS in chronic anovulatory women who have elevated LH and normal ACTH levels. In addition, the finding that the deeper layer of the zona fasciculata, which can also secrete cortisol, contains LH/hCG receptors may also possibly explain the ability of hCG to stimulate cortisol secretion in guinea pig adrenal cells (11).

ACTH is not responsible for increased secretion of adrenal androgens during adrenarche (26). However, which hormones are responsible is not known. Now that we know the zona reticularis contains LH/hCG receptors, it will be interesting to reinvestigate whether LH is responsible for increased secretion of DHEAS, at least in some cases of normal or premature adrenarche. It will be equally interesting to determine whether hCG is capable of affecting DHEAS and cortisol secretion by maternal adrenal glands during pregnancy and the functional relevance of LH/hCG receptors in male adrenal glands.

In summary, we conclude that the steroidogenic cells in the entire zona reticularis and the deeper layer of zona fasciculata contain LH/hCG receptors. These findings may potentially explain increased secretion of adrenal androgens in chronic anovulatory women.

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