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EVIDENCE FOR THE PRESENCE OF ANDROGEN RECEPTORS IN HUMAN LEYDIG CELLS

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Summary—Localization of androgen receptors (ARs) in the human testis Leydig cells was examined with an AR assay and Northern blot analysis. Leydig cells, highly purified on a Percoll gradient, were used for the experiments. AR concentration in the total cell extract containing both the cytosol and nuclear fractions in Leydig cells was measured using [^{3}H]methyltrienolone. ARs in Leydig cells showed a high affinity for [^{3}H]methyltrienolone and the K_d and B_{max} of the receptors were 1.24 nM and 11.7 fmol/mg protein, respectively. Northern blot analysis, using a ^{32}P-labeled full-length human AR complementary DNA (cDNA) detected a 9.5-kb hybridizing band in the total RNA extracted from Leydig cells. These data can be interpreted as evidence of the existence of ARs in human Leydig cells.

INTRODUCTION

There is increasing evidence that testicular steroidogenesis is not influenced only by the classic mechanism of gonadotropin and steroid action. The demonstration of specific androgen receptors (ARs) in Leydig cells [1, 2] is of particular interest in the light of recent studies suggesting that testicular androgen production may be locally autoregulated via ultrashort loop negative feedback mechanisms [3]. Although biochemical techniques have detected ARs in the testicular tissues of various animals [4–8], the exact location has remained unclear, especially in the human testis [9].

In the present study, localization of ARs of the human testis was investigated with AR assays and Northern blot analysis.

EXPERIMENTAL

Materials

Testicular materials consisted of tissues from 10 human testes without severe histologic damage (Johnsen's [10] score count: 9–10), biopsied after informed consent from patients with left varicocele to confirm histologic damage. The age of the patients was 25–34 yr. All had normal serum levels of FSH, LH, prolactin, testosterone and estradiol-17β. None of the patients had received any hormonal treatment before the testicular biopsy. Human hypertrophied prostate tissue and normal urinary bladder tissue used for Northern blot analysis were obtained from a patient with benign prostatic hypertrophy and a patient with bladder cancer, respectively.

Specific chemicals

Human AR complementary DNA (cDNA) [11] was kindly provided by Dr S. Liao and his associates at the University of Chicago (Ill., U.S.A.). [^{3}H]Methyltrienolone (R1881) (87 Ci/mmol) and [^{32}P]deoxyCTP (>6000 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, Mass, U.S.A.). Other chemicals were of the highest grade available.

Isolation of highly purified Leydig cells

Crude interstitial cells were isolated by treatment with collagenase Type I (Sigma, St Louis, Mo., U.S.A.), as described by Grotjan and Steinberger [12]. Dispersed cells were further purified by centrifugation on continuous Percoll gradients with a modification of the method described by Browning *et al.* [13]. The percentage of Leydig cells was determined by histochemical staining for 3β-hydroxysteroid dehydroxylase [14]. The purity of Leydig cells was >95%.

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Measurement of AR concentration in the total cell extract

Freshly-isolated Leydig cells or whole testicular tissues were gently homogenized on ice, together with a high salt homogenization buffer [0.4 mol/l KCl, 20 mmol/l Tris HCl, 10 mmol/l Na_2MoO_4, 10% (v/v) glycerol, 10 mmol/l dithiothereitol] supplemented with 0.2 mg/ml leupeptin and 0.2% (v/v) phenylmethyl sulfonic fluoride, in a glass homogenizer. The homogenates, to which 1 μmol/l dexamethazone was added to inhibit glucocorticoid receptors, were centrifuged at 105,000 *g* for 60 min at 4°C to yield the total cell extract. This fraction was assumed to contain both cytosol and nuclear fractions. To remove the endogenous steroids, the total cell extract was incubated for 30 min at 4°C with a dextran-coated charcoal (DCC) pellet. This pellet was made by centrifuging four times at 2000 rpm for 10 min the sample of DCC solution [10 mmol/l Tris, 1 mmol/l EDTA, 0.5% (v/v) dextran T-70, 0.5% (v/v) charcoal]. After removal of the pellet by centrifugation, aliquots of the samples were incubated with [^{3}H]R1881 (5–80 nmol/l) with and without a 200-fold molar excess of nonlabeled R1881 at 4°C for 18 h. The total incubation volume was 240 μl/tube. Bound and free steroids were separated with the same volume of the DCC solution. After the centrifugation to remove the DCC the radioactivity of the aliquots was measured with a liquid scintillation counter, and the dissociation constant (K_d) and maximum binding capacity (B_{max}) of the receptors were calculated by Scatchard analysis.

Northern blot analysis

Freshly-isolated Leydig cells, whole testicular tissues, hypertrophied prostate tissue and normal urinary bladder tissue were immediately frozen in liquid nitrogen and stored at −80°C until the RNA preparation. RNA was prepared by the LiCl precipitation procedure [15]. RNA gel electrophoresis was then performed as described previously [16], with 50 μg of total RNA applied to each lane. The RNAs were transferred to a Zetabind nylon membrane (Xydex Co., Bedford, Mass, U.S.A.) with the aid of a VACUGENE (LKB, Uppsala, Sweden). RNA blots were probed with ^{32}P-labeled cDNA probes for human AR.

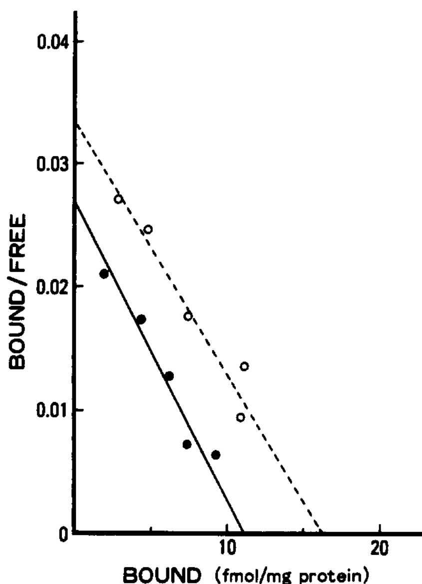

Fig. 1. Specific bindings of [^{3}H]R1881 to the total cell extract obtained from freshly-isolated Leydig cells (●) and whole testicular tissues (○) are plotted according to the Scatchard method. In both types of tissues, only one high affinity binding site was observed. The K_d and B_{max} of the receptors in Leydig cells ($n = 5$) and whole testicular tissues ($n = 5$) are 1.31 ± 0.27 nM and 11.4 ± 1.3 fmol/mg protein and 1.18 ± 0.25 nM and 16.5 ± 1.5 fmol/mg protein, respectively (values are the mean ± SE of 5 samples). The B_{max} of the former was less than that of the latter ($P < 0.05$, *t*-test was used), while the K_d of both receptors was similar (*t*-test was used).

RESULTS

AR assay

Specific bindings of [^{3}H]R1881 to the total cell extract obtained from freshly-isolated Leydig cells and whole testicular tissues are plotted according to the Scatchard method in Fig. 1. In both types of tissue, only one high affinity binding site was observed. The K_d and B_{max} of the receptors on Leydig cells and whole testicular tissues are calculated. The B_{max} of the former was less than that of the latter ($P < 0.05$, *t*-test was used), while the K_d of both receptors was similar (*t*-test).

Northern blot analysis

Figure 2 shows a transfer blot of equal amounts of total cellular RNA from a variety of human tissues, namely purified Leydig cells, whole testicular tissues, hypertrophied prostate tissue and normal urinary bladder tissue, subsequently probed with a full-length cDNA for human AR. Two hybridizing species, one at 9.5 kb and one at 5.0 kb, were detected in RNAs from purified Leydig cells as well as from whole testis and prostate tissue, but not in the RNA from normal urinary bladder tissue.

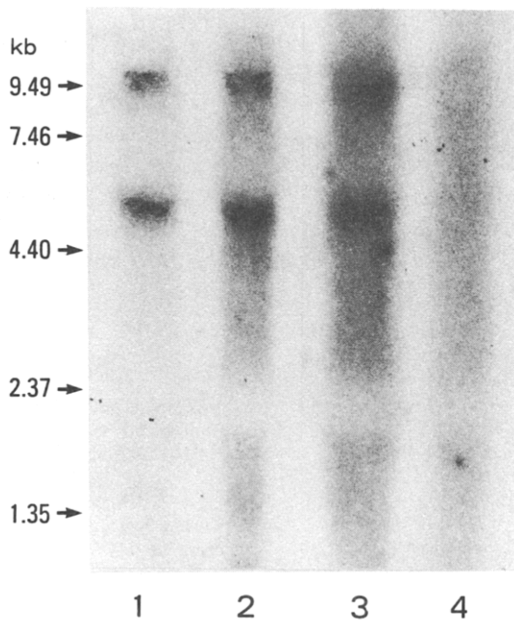

Fig. 2. Northern blot of equal amounts (50 μg) of total cellular RNA from purified Leydig cells (lane 1), whole testicular tissues (lane 2), hypertrophied prostate tissue (lane 3) and normal urinary bladder tissue (lane 4), probed with a ^{32}P-labeled full-length cDNA for human AR. A 9.5-kb hybridizing species was detected in RNAs from purified Leydig cells as well as from whole testis and prostate tissue, but not in the RNA from normal bladder tissue. The intense bands at 5.0 kb are possibly due to nonspecific interaction of the probe with 28S ribosomal RNA.

DISCUSSION

The possible existence of ARs in human Leydig cells was examined with a biochemical AR assay and Northern blot analysis, whereas most previous studies [1, 2] demonstrating the existence of ARs in Leydig cells have used only an AR assay of purified Leydig cells. In our study, Leydig cells were purified on a Percoll gradient, as reported previously [13]. The purity of our cell preparation was so high that it is unlikely that even a small fraction of other contaminating cells could have affected the results of any of our experiments.

In the AR assay the total cell extract containing both the cytosol and nuclear fractions was used to measure AR concentrations, because ARs could be detected in both fractions *in vitro* [2]. We used [^{3}H]R1881 as a radioactive ligand, because R1881 has a high affinity for ARs but not for sex hormone binding globulin (SHBG), and is not enzymatically converted to less active derivatives [17]. Recently, molecular cloning of the cDNA of androgen binding protein (ABP) has shown that ABP is the same protein as SHBG [18]. Therefore, it is unlikely that contaminating SHBG and ABP affected the results of our AR assays. Some reports have mentioned that R1881 could also bind to progesterone receptors [19]. However, Graham *et al.* [9] demonstrated that [^{3}H]R1881 bound mostly to ARs rather than to progesterone receptors, especially in the testis. While the affinity of the AR in purified Leydig cells was similar to that in whole testicular tissues, the B_{max} of the AR in the former was less than in the latter. This means that other types of testicular cells such as Sertoli cells must have ARs as well. ARs have been demonstrated to be present in Sertoli cells [4]. Therefore, this finding is not inconsistent with other reports [7] suggesting the existence of ARs in various types of testicular cells.

The existence of AR mRNA in purified Leydig cells is more convincing evidence that ARs are produced in Leydig cells. The Northern blotting of cellular RNA from purified Leydig cells, whole testicular tissues and hypertrophied prostate tissue yields two bands, one at 9.5 kb and one at 5.0 kb, whereas no spots were detected on the lane of normal urinary bladder tissue. The 9.5-kb band detected in the present study was consistent with AR mRNA in other reports [20, 21] in molecular size. The significance of the other 5.0-kb band is unknown at present. Lubahn *et al.* [21] explained that it was probably due to nonspecific interaction of the probe with 28S ribosomal RNA.

Several studies have suggested evidence for autoregulation of Leydig cell functions in animals [3, 22]. The present study shows the presence of ARs in human Leydig cells and suggests that such autoregulation may also exist in the human testis.

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