INHIBITION OF HEN OVIDUCT ESTRADIOL RECEPTOR BY AURINTRICARBOXYLIC ACID

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1. Introduction

A two-step mechanism for the mode of action of steroid hormones has been proposed [1]. Some recent studies have indicated that the uptake of cytoplasmic progesterone receptor by isolated nuclei can be affected by certain inhibitors [2–5]. Aurintricarboxylic acid (ATA), a triphenylmethane dye, has been shown to interfere with the activities of DNA [6] and RNA polymerases [7]. It has been postulated that ATA combines with the template binding sites of these enzymes [8]. We attempted to investigate if ATA would similarly interfere with the properties like nuclear uptake and DNA binding of estradiol receptor (ER) from hen oviduct. Identification and characterization of ER from chick oviduct has been reported [9,10].

2. Materials and methods

2.1. *Preparation of estradiol receptor*

Oviducts from egg-laying white Leghorn hens were obtained from a local produce company and the receptor was prepared as in [11,12].

2.2. *Nuclear binding assay*

Nuclei were isolated from hen oviduct tissue by the method in [13]. Nuclear assay consisted of incubation of hormone receptor complexes with ATA or buffer followed by the addition of a nuclear aliquot containing about 50 μg DNA [2,13]. DNA was quantitated by the method in [14].

2.3. *Affinity chromatography*

Samples containing ER complexes were chromatographed on short columns of ATP–Sepharose [11,12] or DNA-cellulose [15]. Columns were washed with low salt buffers (Tris 10 mM, 1 mM EDTA, 12 mM thioglycerol (pH 8.0) with 0.01 M KCl) or same buffer containing 1 M KCl. All buffers contained 20% glycerol.

3. Results and discussion

Figure 1 shows our results on nuclear uptake of ER. Binding of ER complexes to isolated hen oviduct nuclei showed a dramatic decrease (~60%) when incubated in presence of 0.5 mM ATA. Comparable results were obtained when lower (0.1–0.2 mM) ATA concentrations were used in several experiments. The steroid binding properties of ER, however, remained intact (data not shown). These results indicate that ATA interferes with the nuclear binding properties of ER, possibly complexing to a site or sites on receptor protein involved in such processes.

A DNA binding site for the steroid receptors has been postulated [15–17]. In our studies, when ER complexes were allowed to mix with DNA-cellulose in a form of slurry with and without a pretreatment of 0.3 mM ATA, the binding of ER to DNA was reduced by about 50% in samples pretreated with ATA. In other studies, ATA has been known to reduce specifically the affinity of RNA directed DNA polymerase for the DNA primer molecule [18]. In addition, ATA has been suggested to combine with the template binding sites of nucleic acid binding proteins [18]. Our results indicate that ATA may also be binding to a DNA binding site of ER molecule, thus blocking interaction with DNA.

Earlier studies have demonstrated an interaction

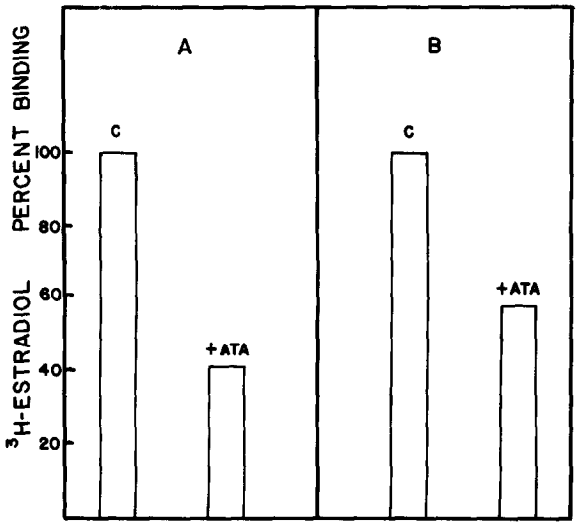

Fig.1. Effect of ATA on the nuclear uptake and DNA binding of ER. (A) Receptor preparation was incubated with 10 nM [^{3}H]estradiol (98.5 Ci/mmol; New England Nuclear) in total vol. 0.5 ml and with 20% glycerol. Samples were then treated with buffer or 0.5 mM ATA. After 2 h at 4°C, 50 μl aliquots of isolated hen oviduct nuclei containing 50 μg DNA were added and tubes mixed gently for 1 h. The nuclear binding assays were performed as in [13]. (B) ER complexes were treated with a 0.5% charcoal suspension to remove free hormone and then incubated with or without ATA (0.3 mM) for 1 h at 4°C. A batch assay was used to monitor DNA binding of ER. Samples were incubated with DNA-cellulose, and the affinity resin was washed with TETG buffer containing 0.01 M KCl and 20% glycerol (pH 8.0). The ER complexes were recovered by stirring the resin with same buffer but containing 1 M KCl. Supernatants, upon centrifugation at 1000 × *g*, were used to measure radioactivity.

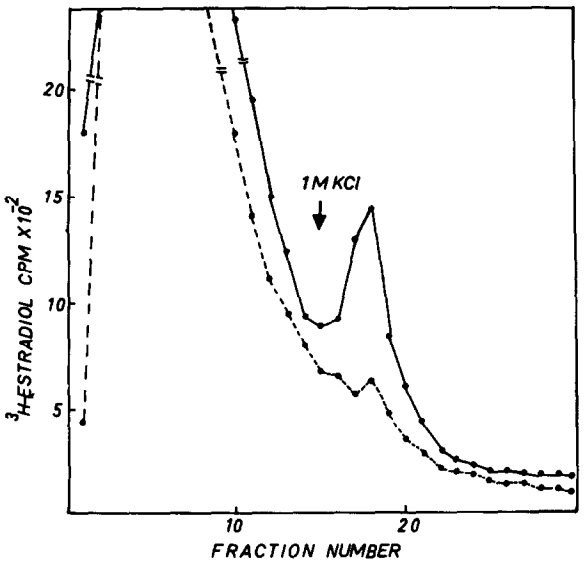

Fig.2. Effect of ATA on binding of ER to ATP–Sepharose. The [^{3}H]estradiol (10 nM) was complexed to ER for 16 h and the complexes were then incubated with their TETG buffer (•——•) or 0.5 mM ATA (•– –•) for 2 h at 4°C. Following this, the entire samples (0.5 ml) were chromatographed on short (1 ml) columns of ATP–Sepharose. Affinity columns were equilibrated with TETG 0.01 M KCl buffer with 20% glycerol and 15 fractions were collected with same buffer. The ER complexes were then eluted with same buffer but containing 1 M KCl. The procedures for column chromatography were essentially same as described earlier [11,12]. The lines at the beginning, prior to the appearance of the peak, represent excess free hormone plus the amount of estradiol receptor complex not retained by the ATP–Sepharose.

between ATP and steroid receptors [11,12,19]. The binding of ATP has been shown to be a property of activated receptor. In the present studies, we demonstrate an interaction of ER from hen oviduct with ATP, which is also sensitive to the presence of low concentrations of ATA (fig.2). A pretreatment of ER complexes with ATA eliminated almost entire binding of ER complexes to ATP–Sepharose. These results support some preliminary observations reporting an interaction of ATP with progesterone receptor which was affected by ATA presence [3,20].

A nucleotide binding site could be closely related to nuclear and DNA binding sites of ER. An interference by ATA in all of the above properties which represent important events in hormone action could be of immense importance. ATA does not destroy the steroid binding characteristics of ER, suggesting a preferential blockage of nuclear events. Efforts are underway to show, by direct binding studies, a binding site for ATA on the receptor molecule.The question, however, remains whether ATA binds to one site and alters different functional parameters of receptor or it binds at different but related sites involved in those processes.

ATA has been used to block the activities of polymerases [6–8]. Similarities between polymerases and steroid receptors have been suggested based on the effects of several compounds which inhibit both poly-

merases and progesterone receptor like ATP, nuclear and DNA binding. Moreover, highly purified progesterone receptor has been shown to catalyze metabolism of ATP to AMP and pyrophosphate (PP_i) by a PP_i-exchange reaction which is a common property of nucleotidyl transferases [21]. These studies are also consistent with an earlier prediction that ER may be a metalloprotein [22]. Incidentally most DNA and RNA polymerases are known to be metal-containing enzymes. Although the mode of action of steroid hormones is still obscure, our studies suggest similarities between polymerases and ER, at least based on the effects of ATA and other inhibitors [2,18,20,21].

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