SYNERGISTIC AND ANTAGONISTIC EFFECTS OF PROGESTERONE AND OESTROGEN ON OESTROGEN RECEPTOR CONCENTRATION AND DNA POLYMERASE ACTIVITY IN CHICK OVIDUCT

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1. Introduction

The daily administration of oestrogen to immature female chickens induces oviduct growth, cell division and the differentiation of epithelial cells into tubular gland cells which synthesize a number of egg-white proteins [1–3]. Simultaneous administration of progesterone antagonizes the oestrogen-induced tissue growth, cell division, tubular gland cell formation and subsequent egg-white protein synthesis [1–4].

The situation is somewhat different when oestrogen-withdrawn immature chickens [2] are treated in a similar manner. Both oestrogen and progesterone are capable of independently inducing the synthesis of ovalbumin in preexisting tubular gland cells. Furthermore, oestrogen and progesterone act synergistically on the synthesis of these proteins although progesterone still antagonizes the oestrogen-induced synthesis of new DNA [2].

Since progesterone-induced inhibition of cytoplasmic oestrogen receptor replenishment has been implicated in progesterone antagonism of oestrogen induced growth in the immature rat uterus [5,6], we have investigated the effect of progesterone on the oestrogen-induced changes in oestrogen receptor concentration and DNA polymerase activity in the oestrogen-withdrawn immature chick oviduct.

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2. Materials and methods

2.1. *Experimental animals*

Four to six week withdrawn immature chickens were used throughout this study. Groups of animals (3–4 animals/group) received intramuscular injections of oestradiol benzoate (1.5 mg/kg body wt) and/or progesterone (3 mg/kg) at zero time. Both hormones were prepared as 10 mg/ml solutions in sesame oil. Some groups of animals, which received oestradiol benzoate alone at zero time, were given an additional single injection of progesterone 6 h, 12 h or 18 h later. Groups of animals were killed at 6 h intervals until 36 h after the initial oestrogen treatment.

2.2. *Preparation of cytoplasmic and nuclear fractions*

Immediately after death the magnum region of the oviduct was removed, placed on ice, and homogenized in a glass–glass tissue grinder in 5 vol. (w/v) medium containing 0.25 M sucrose, 3 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.4 (buffer 1). A portion of the total homogenate was removed for DNA determination [7]. An $800 \times g$ 10 min centrifugation of the homogenate yielded the cytoplasmic fraction and a crude nuclear pellet. Cytosol was obtained by a subsequent $105\,000 \times g$ 60 min centrifugation of the cytoplasmic fraction. Any non-protein bound oestrogen was then removed from the cytosol by a 15 min incubation at

4°C with 1/10th vol. 5% charcoal–0.5% dextran suspension, in 10 mM Tris–HCl, pH 7.4 [8].

Crude nuclei were prepared from the crude nuclear pellet by resuspending the pellet on 10 vol. buffer 1 containing 1% Triton X-100 and centrifuging 800 × g, 10 min. The pellet was washed a further three times with approx. 10 vol. buffer 1 and each wash was followed by a 800 × g, 10 min centrifugation. The final pellet was resuspended in 5 vol. buffer 1 by suction in and out of a pasteur pipette and 0.1 ml aliquots of this suspension were dispensed into tubes for the nuclear oestrogen receptor assay [8].

2.3. Determination of oestrogen receptor concentrations and DNA polymerase activity

The total oestrogen receptor concentrations in cytosol and crude nuclei were assessed by a previously reported [3H]oestradiol exchange technique [8]. Concentrations were expressed as oestrogen binding sites/cell assuming a DNA content of 2.3. pg/diploid genome [9].

DNA polymerase in oviduct cytosol was assayed by a modification of the method of Lynch et al. [11]. Activity was measured by testing the incorporation, in the presence of an exogenous template, of [3H]TMP into an acid-insoluble fraction. The total volume of the assay was 250 μl and contained: Tris 0.1 M, MgCl$_2$ 4 mM, KCl 16 mM, dGTP, dCTP, dATP 0.3 mM, [3H]TTP 0.3 mM, (spec.act. 0.1 Ci/mmol), ATP 2.5 mM, dithiotreitol 5 mM, freshly DNAase-activated calf thymus DNA 50 μg/250 μl, enzyme 180 μl. The pH of the medium was 7.4 or 8.6 according to the fraction tested. Activity was assayed by incubating equivalent aliquots at 37°C for various times until 10 min and stopped by adding 0.5 ml bovine serum albumin (1 mg/ml) and 0.75 ml cold perchloric acid (PCA) 0.5 N. The precipitate was centrifuged 700 × g, 5 min and washed twice with 5% PCA, then resuspended in 2 ml 0.3 N sodium hydroxyde and incubated 1 h at 37°C. The DNA was reprecipitated by adding 0.2 ml PCA 70% and the precipitate centrifuged and washed 3 times with PCA 5%. The final pellet was hydrolyzed 20 min at 70°C in 1 ml PCA 0.5 N and an aliquot of the supernatant was used for counting, another sometimes to check DNA content. DNA polymerase activity was expressed as dpm incorporated/min/mg DNA contained in 1 ml initial tissue homogenate.

3. Results

Separate studies on the kinetics of receptor and DNA polymerase changes are reported elsewhere [10,12]. The data shown here were obtained with a single set of animals, in order to confirm the correlations which appeared from the previous independent experiments.

3.1. Effect of a single injection of oestradiol benzoate (1.5 mg/kg) on oestrogen receptor concentration and DNA polymerase activity

The administration of a single dose of oestradiol benzoate resulted in translocation of a portion of the oviduct oestrogen receptor sites from the cytoplasm to the nucleus during the first 6 h after injection (fig.1). This initial depletion of cytoplasmic receptor sites was followed by a period of receptor replenishment
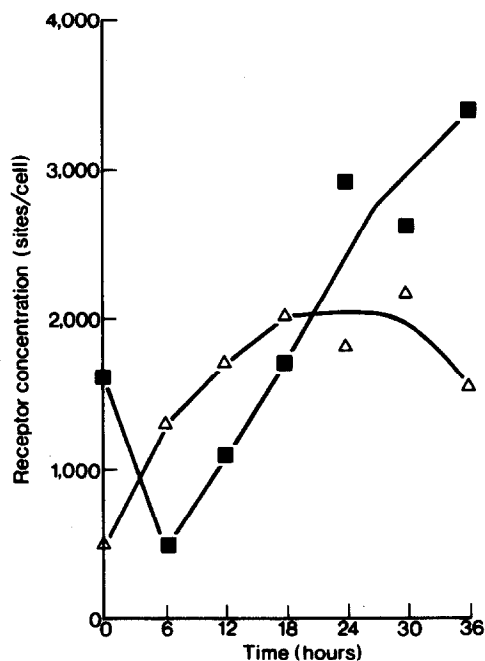

Fig.1. Oestrogen receptor concentrations in magnum cytoplasm and nuclei following a single injection of oestradiol benzoate to oestrogen withdrawn chickens. Groups of chicks received a single intramuscular injection of 1.5 mg oestradiol benzoate/ kg body wt and were sacrificed at the times indicated on the graph. The concentrations of cytoplasmic (■———■) and nuclear (△———△) oestrogen receptor sites were assessed as described in Materials and methods.
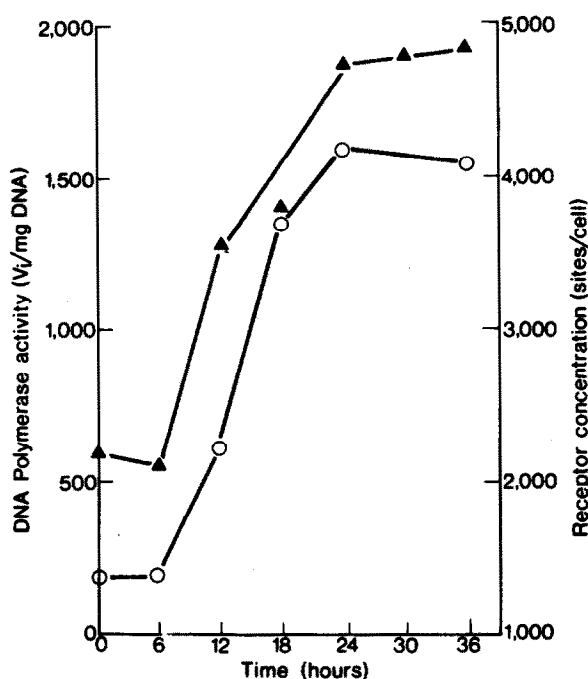
Fig.2. Changes in total cellular oestrogen receptor site concentration and DNA polymerase activity in chick magnum following a single injection of oestradiol benzoate to oestrogen withdrawn chickens. Groups of chicks received a single intramuscular injection of 1.5 mg oestradiol benzoate/kg body weight and were sacrificed at the times indicated on the graph. Total cellular oestrogen receptor site concentrations (▲———▲) were calculated from the data presented in fig.1. DNA polymerase activity (o - o) was measured as described in Materials and methods.

such that cytoplasmic receptor levels reached pre-injection levels at 18 h and subsequently increased to a maximum of 220% of control at 36 h. Nuclear oestrogen receptor concentrations continued to increase after 6 h to reach a maximum, at 30 h, which was approximately 400% of the pre-injection control value (fig.1). A decline in the concentration of nuclear receptor sites was observed between 30 h and 36 h. The total number of cellular oestrogen receptor sites did not change during the first 6 h after injection. During the next 18 h a 2.3-fold increase was apparent with little further change between 24 h and 36 h (fig.2).

The oestrogen-induced changes in DNA polymerase activity were similar to those of total oestrogen receptor concentration. After a lag period of 6 h, DNA polymerase activity increased dramatically for 12–18 h.

Thereafter the rate of increase was diminished, and at 36 h the enzyme activity was still approximately 8 times that seen in non-injected control animals (fig.2).

3.2. Effect of progesterone on oestradiol benzoate induced changes in oestrogen receptor concentration and DNA polymerase activity

The injection of progesterone (3 mg/kg) at the same time or at 6 h, 12 h or 18 h after a single injection of oestradiol benzoate, resulted in inhibition of cytoplasmic oestrogen receptor replenishment during the ensuing 6 h period. This reduction in cytoplasmic receptor versus animals receiving oestrogen alone, was accompanied by a reduction in nuclear oestrogen receptor concentration, total cellular oestrogen receptor site concentration, and DNA polymerase activity during the same 6 h period (fig.3). Subsequently all the measured parameters increased in the progesterone treated animals and by 18 h after progesterone treatment both nuclear receptor concentration and DNA polymerase activity exceeded the corresponding values in animals receiving oestrogen only (fig.3).
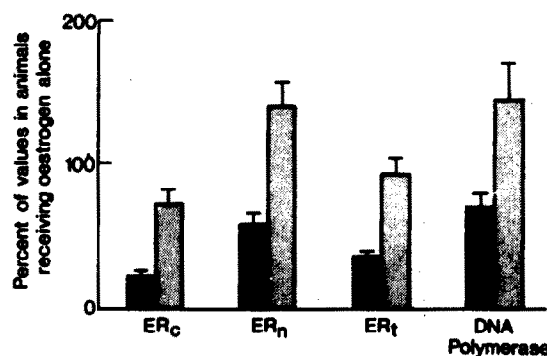

Fig.3. Effect of progesterone on oestrogen induced changes in oestrogen receptor concentrations and DNA polymerase activity in chick magnum. Groups of chickens were given a single injection of progesterone (3 mg/kg) at the same time, 6 h, 12 h, or 18 h after a single injection of oestradiol benzoate (1.5 mg/kg). Animals were sacrificed at 6 h or 18 h after each progesterone treatment and the concentrations of cytoplasmic (ER_c), nuclear (ER_n) and total (ER_t) oestrogen receptor sites as well as DNA polymerase activities were assayed as described in Materials and methods. The data are presented as percentages of the corresponding values in animals which received oestradiol benzoate only. The open bars represent the mean ± SD values 6 h after progesterone treatment while the hatched bars represent the corresponding parameters 18 h after progesterone treatment.

3.3. Effect of a single injection of progesterone (3mg/kg) on oestrogen receptor concentration and DNA polymerase activity

The administration of progesterone alone to oestrogen withdrawn chicks resulted in an initial decline in cytoplasmic oestrogen receptors during the first 3 h. This decline was followed by a period of cytoplasmic receptor accumulation and at 24 h the concentration was approximately 170% of the pre-injection control value (fig.4). Nuclear receptor levels increased gradually to approximately 200% of control at 15 h and thereafter declined slightly, but were still significantly above control values at 24 h. These changes in oestrogen receptor concentration and subcellular distribution were associated with a 2-fold increase in DNA polymerase activity at 24 h, as previously observed [10].

4. Discussion

The data presented here illustrate that a single injection of oestradiol benzoate to oestrogen withdrawn immature chickens induces significant changes in the concentration and subcellular distribution of oestrogen receptor sites and a 8-fold increase in magnum DNA polymerase activity (figs 1 and 2). Administration of progesterone at 6 h, 12 h or 18 h after oestrogen treatment resulted in short term (6–12 h) inhibition of the oestrogen induced increase in DNA polymerase activity (fig.3). Following this period of inhibition, the rate of increase in DNA polymerase activity was greater in progesterone treated animals and by 18 h after progesterone treatment the enzyme activity had exceeded that in chickens receiving oestrogen alone (fig.3). At all times nuclear oestrogen receptor levels were closely correlated with DNA polymerase activities which suggested that nuclear receptors may be intimately involved in the control of DNA polymerase synthesis. We have previously shown that the changes in enzyme activity cannot be explained, either by changes in the affinity of the enzyme for its substrates, or by changes in its affinity for the exogenous DNA template [10]. It therefore seems reasonable to postulate that the oestrogen induced increase in DNA polymerase activity is due to increased synthesis of the enzyme protein.

The oestrogen receptor kinetics presented in fig.1 are typical of tissues which respond to oestrogen by increased tissue growth and cell division, e.g., immature rat uterus and chick oviduct [8,13–15]. Prior to injection the majority of cellular receptors are located in the cytoplasmic fraction. Administration of oestrogen results in translocation of a portion of cytoplasmic receptors to the nucleus during the first hour [8,12–15]. Translocation is followed by cytoplasmic receptor replenishment which probably involves both return of receptor from the nucleus and synthesis of new cytoplasmic receptor [13]. Certainly new receptor activation and/or synthesis must occur since an increase in total receptor sites/cell occurs after 6 h and by 24 h cytoplasmic receptor levels invariably exceed those in non-injected control animals [8,13,14].

Progesterone has the ability to both inhibit and augment the concentration of oestrogen receptor sites in the chick oviduct. Shortly after progesterone administration the level of cytoplasmic and total
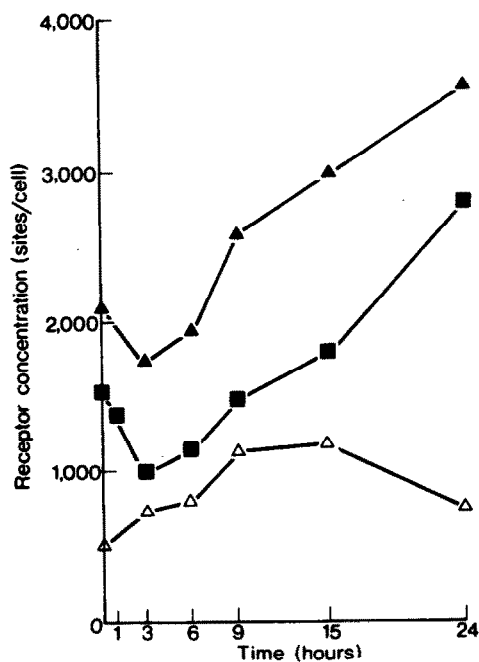

Fig.4. Oestrogen receptor concentrations in magnum cytoplasm and nuclei following a single injection of progesterone to oestrogen withdrawn chickens. Groups of chickens received a single intramuscular injection of 3.0 mg progesterone/kg body weight and were sacrificed at the times indicated on the graph. The concentrations of cytoplasmic (■———■), nuclear (△———△), and total (▲———▲) oestrogen receptor sites were assessed as described in Materials and methods.

oestrogen receptor sites/cell declined (figs 3 and 4). This may be explained by the previously reported inhibitory effect of progesterone on the oestrogen induced synthesis of new cytoplasmic oestrogen receptor [5,6,16]. The initial decline was followed by receptor accumulation (figs 3 and 4) and by 18–24 h after progesterone administration, oestradiol receptor levels were significantly greater than those in non-injected controls, as in uterus endometrium [16]. However, while nuclear oestrogen receptor levels at this time were always greater than the corresponding values in animals receiving oestrogen alone, the concentration of cytoplasmic receptor was invariably lower than that in oestrogen treated animals and the tissue would thus be rendered less receptive to subsequent administration of oestrogen [14].

The situation realized by a single injection of progesterone in withdrawn chicks is difficult to interpret. In these prepuberal animals, there might be a low level of circulating oestradiol, and the initial decrease of cytoplasmic receptor brought about by progesterone may be qualitatively similar to the situation reported for the first 6 h after progesterone in oestrogen-treated animals. The late increase of nuclear oestradiol receptor could eventually be due to a metabolic transformation of injected progesterone into oestrogen, although this does not seem probable because of the low level of presumed transformation and of the lack of concurrent decrease of cytosol receptor.

These data may contribute to explain partially aspects of the synergy and antagonism of oestradiol and progesterone, both active upon oviduct sensitive cells during the ovulatory cycle [17].

Acknowledgements

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