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ESTROGEN-LIKE EFFECTS OF COMBINED DEXAMETHASONE AND TAMOXIFEN IN THE CHICK OVIDUCT

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SUMMARY : The effects of dexamethasone alone on withdrawn chick oviduct weight, DNA, protein content and progesterone receptor concentration were barely detectable, whereas ovalbumin and conalbumin synthesis were increased. When dexamethasone and tamoxifen were combined, a marked increase of total proteins, including egg white proteins, DNA and wet weight occurred. Progesterone receptor also was increased. The most striking result was the stimulation of DNA polymerase-α activity by combined dexamethasone and tamoxifen, whereas either compound was completely ineffective.

The nonsteroidal triphenylethylene antiestrogen tamoxifen does not influence the growth parameters, the level of progesterone receptor, or the synthesis of egg white proteins in the chick oviduct (1). We had previously reported that the combined administration of tamoxifen and progesterone to estrogen primed, withdrawn chicks potentiated the effects of progesterone on egg white protein synthesis and produced growth promoting properties (2, 3).

Some effects of glucocorticosteroids on the chick oviduct resemble those produced by progesterone. They induce ovalbumin and conalbumin synthesis in withdrawn chicks and the magnitude and kinetics of the responses obtained are comparable to those obtained with estrogen or progesterone (4). Glucocorticosteroids act synergistically with estrogen. In appropriate conditions the effects of dexamethasone and of progesterone are additive (5). The effects of glucocorticosteroids on the growth of withdrawn chick oviducts have not yet been reported, and the results of antiestrogen-glucocorticosteroid combinations have not been described. In the present work, we have used the measurement of DNA-polymerase-α activity as a sensitive parameter of the oviduct's growth potential. Several reports had previously described the increase of this enzyme activity during estrogen stimulated growth of chick oviduct (6) and rat uterus (7).

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MATERIALS AND METHODS

Experimental animals : Newborn chicks were primed with estradiol benzoate and withdrawn for 4-6 weeks. After this period, they were reinjected in the upper leg muscle with the appropriate test compounds, as indicated in the Results section. Animals were killed 18 h after the last injection. The oviducts were removed, fragments of each oviduct magnum were pooled, and the relative rates of ovalbumin and conalbumin synthesis were measured as previously described (2, 8).

Progesterone receptor measurement : The technique utilized allowed measurement of total (filled and unfilled) PR sites in the cytosol, as reported in detail by Bayard et al. (9), with modifications. The cytosol was stripped of endogenous steroids by dextran-charcoal adsorption, then samples were incubated at 0°C for 15 h followed by 25°C for 2 h with ^{3}H-progesterone (range 0.5-10 nM) in presence of 0.5 µM cortisol. Unbound ligand was separated by dextran-coated charcoal adsorption and the equilibrium binding constants were determined by Scatchard analysis, using the Rosenthal correction for non saturable binding (10). The results were expressed in sites/cells, assuming a DNA concentration of 2.5 pg per cell of chick oviduct (6).

DNA-Polymerase-α : Oviducts were homogenized in 5 vol of chilled buffer containing 20 mM Tris HCl pH 7.5, 0.5 M KCl, 2 mM dithioerythritol, and 0.5 % Triton X 100. The homogenates were sonicated for 20 sec using a Branson I-22 sonicator and kept at 0°C for 60 min. The 105,000 g supernatant was used for DNA Polymerase-α assay according to Bertazzoni et al. (11). The results were expressed in pmol thymidine triphosphate incorporated per min per mg protein.

Measurement of DNA and proteins : Protein and DNA were measured in samples of homogenates and/or cytosol using the techniques of Bradford (12) and Burton (13), respectively.

RESULTS

Progesterone receptor : The concentrations of cytosol PR were measured in withdrawn oviducts (control) and after 90 h of secondary stimulation with dexamethasone and tamoxifen, alone or combined. Estradiol benzoate was given for comparison (Figure 1). Results were expressed in % of control (14,800 $\pm$ 600 sites/cell). Estradiol benzoate increased PR concentration more than 4 fold. Tamoxifen tended to decrease PR 20 % below control value and counteracted completely the effect of estradiol benzoate. Dexamethasone was ineffective ; however when combined with tamoxifen a slight (20 % above control) although statistically significant increase of cytosol PR occurred ($p < 0.5$).

Weight, DNA and protein content : The effects of dexamethasone, injected alone or combined with tamoxifen, were determined after 18, 24 or 90 h. Estradiol benzoate was given for comparison. Tamoxifen alone had no effect on oviduct growth parameters (data not shown) (3). Dexamethasone alone had minimal effects on wet weight, DNA and protein content (Figure 2). Tamoxifen

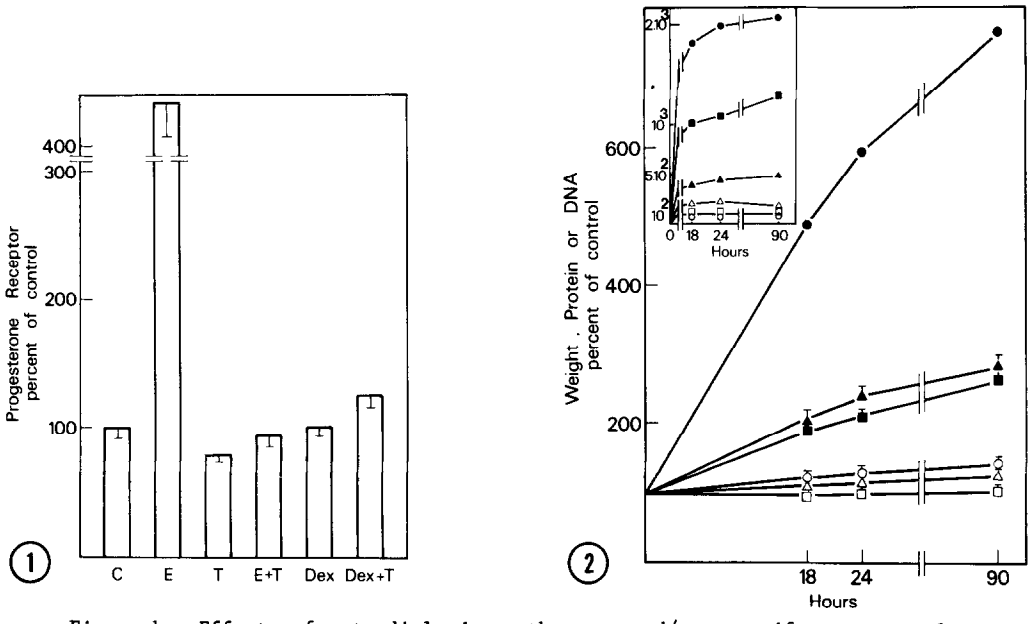

Figure 1 : Effects of estradiol, dexamethasone, and/or tamoxifen on cytosol PR.

Withdrawn chicks received daily injections of estradiol benzoate (E, 1 mg/kd/d), tamoxifen (TAM, 10 mg/kd/d), or dexamethasone (DEX, 2 mg/kg/d) for 4 d and were killed 18 h after the last injection. E and DEX have also been combined with TAM. Results are expressed in % of control (C) PR concentration (14,800 ± 600 sites/cell, mean ± sd, n = 3).

Figure 2 : Effects of estradiol, dexamethasone and/or tamoxifen on oviduct weight, DNA, and proteins.

Withdrawn chicks were treated as reported in the legend of Fig. 1, and sacrificed 18, 24, or 90 h after the first injection.

Open symbols correspond to DEX alone (2 mg/kg) and closed symbols to DEX + TAM (10 mg/kg). Results are expressed in % of control.

●: Protein (5.5 ± 0.2 mg per oviduct ; ▲: DNA (1.26 ± 0.1 mg per oviduct ; ■: wet weight (76 ± 4 mg per oviduct).

Insert : Estradiol benzoate (1 mg/kg) has been injected instead of DEX. The same symbols are used.

completely counteracted the effects of estradiol benzoate (Figure 2, Insert). On the contrary, combined dexamethasone and tamoxifen increased the protein content of chick oviduct more than 7 fold, and DNA content more than 2 fold after a 90 h treatment.

The stimulatory effects of dexamethasone on the relative rates of ovalbumin and conalbumin synthesis were also greatly potentiated by tamoxifen. A representative experiment is reported on Table 1. A more detailed report will be published elsewhere (5).

TABLE 1

TREATMENT	OVALBUMIN	CONALBUMIN
Control	0.2	0.8
Tamoxifen	0.2	1.6
Dexamethasone	2.8	4.4
Tamoxifen + Dexamethasone	7.2	7.8

Relative rates of ovalbumin and conalbumin synthesis in chick oviduct. Withdrawn chicken received daily injections of tamoxifen (10 mg) and dexamethasone (2 mg) alone or combined for 90 h. Results are expressed in % of total protein synthesis.

DNA Polymerase-α : The enzyme activity has been measured in chick oviduct extracts 18, 24 and 90 h after the onset of treatment with dexamethasone and tamoxifen, alone or combined. Estradiol benzoate was given for comparison. DNA polymerase activity was unchanged by dexamethasone and tended to decrease under tamoxifen (Figure 3). Nevertheless, combined tamoxifen and dexamethasone increased DNA polymerase-α activity almost 2 fold. The

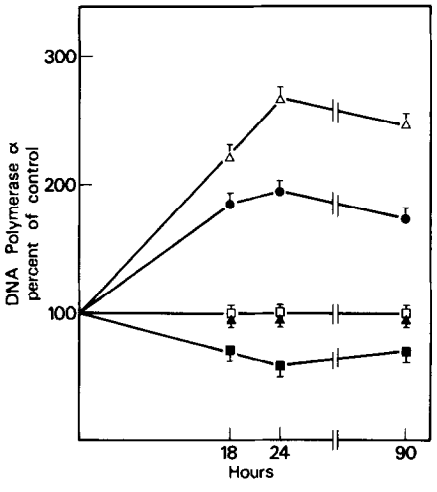

Figure 3 : Effects of dexamethasone or/and tamoxifen, and of estradiol benzoate on DNA-polymerase-α activity.

Withdrawn chicks were treated as reported in the legend to Fig. 1, and sacrificed 18, 24, or 90 h after the first injection.

Open symbols correspond to control (□), DEX (2 mg/kg) (○) or estradiol benzoate (1 mg/kg) (△). Closed symbols correspond to treatments with tamoxifen alone (10 mg/kg), (■) or combined with dexamethasone (●) or with estradiol benzoate (▲). Results are expressed in % of control DNA polymerase-α activity : 14.7 ± 0.4 pmol TTP/min/mg protein.

time-courses of DNA polymerase-α stimulation by estradiol benzoate or by dexamethasone + tamoxifen were similar with a maximum at 24 h.

DISCUSSION

The effects of dexamethasone on egg white protein and mRNA synthesis in withdrawn chick oviduct have been previously reported. Most of the published work utilized magnum explant organ cultures (4, 14, 15). The effects of dexamethasone *in vivo* were reported by Hager et al. after 3 or 10-12 d of withdrawal, a single 5 or 1 mg dose of dexamethasone, and a short time interval (1-8 h) (4). These authors concluded that glucocorticosteroids can mediate an induction of the major egg white proteins with kinetics and dose-response relationships similar to those observed with estrogen or progesterone. Using rather different experimental protocols, as concerns the duration of withdrawal (4 to 6 weeks) the dose of dexamethasone injected (2 mg) and the time interval (18-90 h), we confirm that dexamethasone increases the relative rates of ovalbumin and conalbumin synthesis.

However, the present work was essentially undertaken to compare the chick oviduct's growth control by dexamethasone alone, or combined with tamoxifen. The effects of dexamethasone alone on oviduct weight, DNA or protein content, and progesterone receptor were barely detectable, even after 4 d of stimulation. When dexamethasone and tamoxifen were combined, a marked increase of proteins, DNA and wet weight occurred. Progesterone receptor was slightly but significantly increased. The most striking result was the stimulation of DNA polymerase-α activity by combined dexamethasone and tamoxifen whereas either compound was completely ineffective if not inhibitory. Similar synergistic results had been previously reported with tamoxifen and progesterone, although dexamethasone alone is much less effective than progesterone on the increases of weight, DNA, and proteins (3). On the contrary, it has been reported that dexamethasone exerts an antagonistic action or the growth of quail oviduct stimulated by high doses of estradiol benzoate (16).

In mammalian species, tamoxifen, administered in vivo displays by itself both estrogenic and antiestrogenic activities for undefined reasons (17). In the withdrawn chick, contrary to the effects of tamoxifen alone (1), the combinations of tamoxifen with progesterone (3) or dexamethasone produce oviductal response, which are qualitatively reminiscent of those produced by estradiol (18). Of particular importance in this respect are the parameters related to organ growth, in particular DNA polymerase-α activity. The underlying molecular mechanisms which can explain the estrogen-like effect of tamoxifen when injected with dexamethasone are completely unknown.

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