RETARDED ADMINISTRATION OF AN ANTAGONIST STOPS STEROID HORMONE ACTION

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Received September 8,1977

Summary

Retarded (up to 6 h) administration of tamoxifen, an estrogen antagonist, was found to inhibit the estradiol benzoate induced responses of the chicken magnum (tissue growth, accumulation of cytoplasmic progesterone receptor and of total cellular estrogen receptor, rate of ovalbumin synthesis) measured at 24 h post-estrogen. This inhibition is apparent only after 4 h following the administration of tamoxifen. These results exclude long-lived intermediary component(s) necessary and sufficient for expression of the estrogenic responses, but are compatible with the involvement of such intermediary component(s) having relatively short half-life.

Introduction

The current models of the mechanism of action of steroid hormones assume entry into the cell, binding to a cytoplasmic receptor and translocation of the hormone-receptor complexes into the nucleus, where take place changes of the genetic expression, especially at the transcriptional level (1,2).

The chick oviduct has proved to be an important system to study estradiol action. Estradiol induces in the magnum portion of this organ differentiation, growth and specific protein synthesis

Administered together with estradiol, tamoxifen acts as a very potent and "pure" antiestrogen in the chick oviduct system (3). In this work we have used it in order to examine effects of its administration at various time-intervals after the estrogen.

ISSN 0006-291X

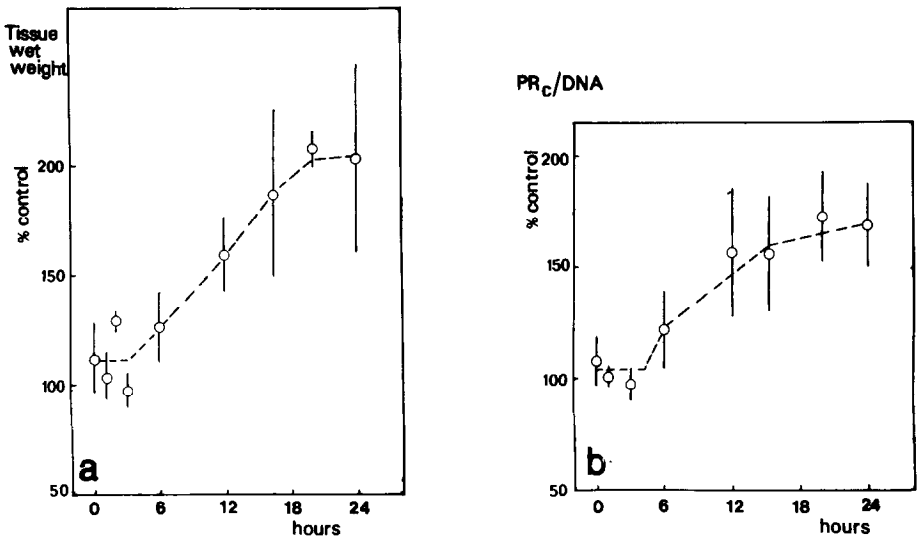

Figure 1 :.
The chickens (groups of 3) were injected with 1 mg estradiol benzoate/kg at time 0 and with 10 mg tamoxifen/kg at times indicated. The control chickens had no treatment. All chickens were killed 24 h after the estrogen, the magnum portion of the oviduct was excised, weighed and homogenized in a Tris (10 mM) - HCl buffer (pH 7.4) containing 0.25 M sucrose and 3 mM $MgCl_2$. The low-speed (800x10 gxmin) cytoplasmic fraction was tested for the cytoplasmic progesterone receptor (PRc) concentration as described previously (6). The results (mean ± s.d. of 2-6 independent experiments) are expressed in terms of % of the control chickens : a. wet weight of the magnum ; b. cytoplasmic progesterone receptor concentration related to the DNA content of the tissue.

Materials and Methods

In order to induce the development of the responsive tubular gland cells (4,5), the chickens (7 days old) were estrogen primed with estradiol benzoate (E_2B, 1 mg/chicken for 10 days) and subsequently withdrawn for 5 to 6 weeks. One mg of E_2B/kg in propylene glycol was given i.m. as secondary estrogen stimulation ; tamoxifen (10 mg/kg) was injected simultaneously or at various time intervals afterwards. The chickens were sacrified by decapitation, magnum portion of the oviduct was excised, weighed and homogenised in the sucrose (0.25 M)-$MgCl_2$(3 mM)-Tris (10 mM) buffer, pH 7.4. The concentration of cytoplasmic progesterone receptor and of cytoplasmic and nuclear estrogen receptors were determined as described previously (6,7). Alternatively, the tissue was cut in pieces and incubated for 1 h at 37°C in the Hanks' balanced salt medium containing 25 µCi/ml of ^3H-labeled amino acids mixture (Radiochemical Centre, Amersham, England) and subsequently homogenised in a Tris (10 mM)-EDTA (1.5 mM) buffer, pH 7.6. The cytosol fraction was prepared and the total protein (TCA-precipitable) and ovalbumin (antibody-precipitable) radioactivity determined according to Palmiter (8).

DNA was assayed by the diphenylamine reaction (9).

Table 1 : Effect of simultaneous and retarded administration of tamoxifen on the augmentation of estrogen receptor concentration induced by estradiol benzoate.

The chickens (groups of 3) were injected with 1 mg estradiol benzoate (E_2B)/kg at time 0 and with 10 mg tamoxifen (Tam)/kg at the same time or 6 h afterwards. The chickens were sacrificed at 24 h after the estradiol administration, the magnum tissue was excised, fractionated and the cytoplasmic (800x10 gxmin) and nuclear estrogen receptor (ER) concentration was determined as described previously (7). The results were related to the DNA content of the tissue. The data shown represent the means ± s.d. of 4 independent experiments.

Treatment	Total ER	Distribution (% total)	
	% control	Cytoplasmic	Nuclear
None	100	66	34
E_2B at time 0	157±16	51	49
E_2B at time 0 Tam at time 0	92± 9	24	76
E_2B at time 0 Tam at time 6h	99± 6	28	72

Results

a. Tamoxifen effects on the 24 h estrogen responses

The estrogen-induced response in terms of wet weight (Fig. 1a), DNA content (data not shown) and the concentration of progesterone receptor (Fig. 1b), a specific cellular protein, were virtually abolished when tamoxifen was given up to 3 h after the estrogen and by more than 70 % when the drug was administered at 6 h (non significant differences from controls). Moreover, the oestradiol-induced increase of the estradiol receptor concentration (another marker of estrogen action) was also abolished (Table 1). The

Table 2 : Effect of retarded tamoxifen injection on the rate of ovalbumin synthesis at 24 h after estrogen stimulation.

The chickens (groups of 2) received 1 mg estradiol benzoate/kg (E_2B) at time 0 and 10 mg/kg of tamoxifen as indicated. The control chickens (C) were not treated by either E_2B or tamoxifen. The rate of ovalbumin synthesis was measured by the procedure of Palmiter (8).

tamoxifen injected at time	ovalbumin synthesis (% total) Exp. 1	Exp. 2
C	0.24	0.15
0h	0.34	0.38
2h	0.67	0.50
6h	0.71	0.52
22h	-	6.23
no tamoxifen	7.26	7.76

relative rate of ovalbumin synthesis which has been shown to reflect the amount of specific mRna of this protein (8) was also inhibited ($>$90 %) by injection of tamoxifen up to 6 h after estrogen (Table 2).

However, tamoxifen injected at 12 h or later after the estrogen did not diminish significantly the magnitude of the cumulative estrogenic responses measured at 24 h (weight, DNA, cytoplasmic progesterone receptor), suggesting that its mode of action is based on inhibition of estrogen induced processes rather than on rapid destruction of their final products. The same conclusion follows from the experiment where the rate of ovalbumin synthesis was determined in the magnum tissue of chicken given tamoxifen at 22 h after the estrogen and killed 2 h later (Table 2).

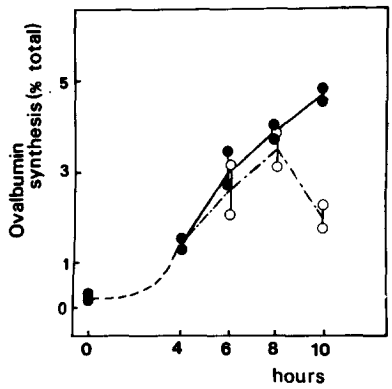

Figure 2 :
Chickens (groups of 2) were injected with 1 mg estradiol benzoate/kg at time 0. Three groups were subsequently injected with 10 mg tamoxifen/kg at time 4 h. The chickens were sacrificed at times indicated and the rate of ovalbumin synthesis was measured in vitro (8). The results shown represent two independent experiments. Full circles : estradiol benzoate injected at time 0 ; open circles : estradiol benzoate injected at time 0 and tamoxifen injected at time 4 h.

b. Time-course of tamoxifen inhibition of the estrogen-induced ovalbumin synthesis

In this set of experiments, tamoxifen was given at 4 h post-estradiol and the rate of ovalbumin synthesis was determined at 4, 6, 8 and 10 h after the hormone. As indicated in Fig. 2, during the first 4 h following tamoxifen administration the rate of ovalbumin synthesis augmented in a similar manner as in the chicken which did not receive the antiestrogen. It is only after that there was a significant difference between the groups.

Discussion

The results of the experiments where E_2B injected to the withdrawn chickens was followed by tamoxifen administration at various time intervals and the response was evaluated at 24 h after the estrogen indicated that the first (approx. 6) hours of estradiol

exposure, which cover the "lag" period of steroid hormone action (10,11), do not lead to the formation of long-lived intermediary product(s) or change(s) that would be sufficient to mediate all the subsequent processes to develop the full response to 1 mg of estradiol benzoate at 24 h.

However, the delay in manifestation of the inhibitory action of tamoxifen observed in the second set of experiments (Fig. 2) is compatible with the hypothesis of short-lived intermediary component(s) mediating hormone action, such as "key intermediary protein" (12) or receptor-ligand-productive site structure evoked in the "receptor translocation" hypothesis (11). Alternatively, the delay in the manifestation of the inhibitory action of tamoxifen may have been due to the rate of access of the antagonist to its target sites within the cells.

In our experimental model, prior to secondary estrogen stimulation, most of the estrogen receptor is found in the cytoplasmic fraction (3,7). Estradiol benzoate leads to a rapid (less than 1 h) translocation of the larger part of the cytoplasmic receptor pool to the nucleus. The nuclear receptor level remains high till about 16 h post-estrogen (even increasing, probably due to protein synthesis, between 6 and 16 h). Only after 16 h new redistribution of the estrogen receptor occurs and at 24 h the receptor is again predominantly found in the cytoplasm. When the chickens are treated by tamoxifen alone, there is also translocation, but the increased nuclear (and correspondingly decreased cytoplasmic) receptor concentrations persist without change throughout at least 24 h (3). The data presented in Table 1 show that when both tamoxifen and estradiol were injected (at the same time or not), 24 h after the estrogen administration the estrogen receptor was found predominantly in the nucleus. These data favor the hypothe-

sis that the persistence of active estradiol-receptor complexes in the nucleus is necessary in order to build up the 24 h estrogenic response ; tamoxifen may replace estradiol and switch off the action of the receptor by converting it into an inactive form.

We propose that when an antagonist such as tamoxifen is administered within several hours after the hormone (in this case estradiol), it replaces progressively the agonist molecules at the nuclear receptor binding sites level, rendering the receptor-ligand complexes inactive, and inhibiting the immediately following step (i.e. the one "executed" by the receptor-estradiol complexes) of estrogen action. This experimental model may provide a valuable tool for identifying the role of receptor in the cell.

Acknowledgements

We thank M.C. Lebeau and M.G. Catelli for their collaboration. The gift of tamoxifen by Dr. A.L. Walpole is gratefully acknowledged. This work was partially supported by the Délégation Générale à la Recherche Scientifique et Technique and the Ford Foundation.

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