The effects of glucocorticoids on thymidine kinase and nucleoside phosphotransferase during development of chicken embryo retina

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Thymidine kinase in chick embryo retina reaches its highest values on the 8–10th day of development, then declines reaching the lowest value at hatching. The rate of DNA synthesis essentially follows this activity while, in contrast, nucleoside phosphotransferase increases progressively during development. Glucocorticoids at 5×10^{-6} M lower the level of thymidine kinase in isolated retinas of chick embryo. The most effective steroid was hydrocortisone. The effect was observed in retinas from 8–18-day-old chick embryo and, except on the 8th day, was always of the same magnitude. We suggest that a glucocorticoid can be the natural factor responsible for the marked fall in thymidine kinase during development. Brief periods of exposure to steriods increase nucleoside phosphotransferase activity in isolated chick embryo retinas. When the exposure was longer than 3 h this activity was also clearly decreased. We conclude that other factors are responsible for the natural increment which occurs for this activity during development.

Thymidine kinase *Glucocorticoid* *Development* *Retina* *Nucleoside phosphotransferase*

1. INTRODUCTION

Many reports indicate that glucocorticoids decrease the level of thymidine kinase in growing tissues and in cells with high rates of DNA synthesis. The authors in [1] found that thymus involution, initiated by hydrocortisone treatment, was accompanied by a marked decrease of thymidine kinase activity and that hydrocortisone also decreased the level of thymidine kinase in erythropoietic organs, fetal liver and neonatal spleen. In adipose tissue of hypophysectomized male rats, simultaneous injections of cortisone prevented the stimulation of thymidine kinase by growth hormone [2]. Finally, the growth of HeLa cells in the presence of dexamethasone resulted in a marked inhibition of exogenously added thymidine incorporation into DNA [3].

Thymidine kinase plays a key role in the 'salvage pathway' of thymidilate synthesis and its activity has been correlated with the production of DNA. Nucleoside phosphotransferase, found in tissues of the embryo and adult chicken [4,5], is another activity which can synthesize thymidilate. It is likely that also this enzyme, at least in certain circumstances, plays a role in the salvage pathway of thymidine.

We determine here the changes in activities of both thymidine kinase and nucleoside phosphotransferase during the development of chick embryo retina. Further we intend to define whether glucocorticoids may be responsible for the changes in enzyme activities.

2. MATERIALS AND METHODS

Glucocorticoids were supplied by Sigma St Louis. Radioactive thymidine was obtained from Sorin, Saluggia. Anion exchange resin AG 1-X8 (200–400 mesh, formate form) was obtained from Bio-Rad, California. Adenosine 5′-triphosphate and 2′-deoxyuridine 5′-monophosphate were supplied by Calbiochem-Behring, San Diego.

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Neural retinas were dissected as in [6] from chick embryos incubated for 8, 9, 10, 12, 14, 16 and 18 days. Individual retinas were placed in 4 ml of Tyrode's solution and incubated at 37°C for various times on a rotary shaker equilibrated with a 5% CO_2–air mixture. Steroids were prepared in stock solutions of 10^{-3} M in absolute ethanol and stored at −20°C prior to use. Usually 20 μl of each steriod solution were added to the incubation medium. The final concentration of steroids was 5×10^{-6} M. In non-steroid-treated controls 20 μl of ethanol were added although this aliquot has no influence on the activities studied. After incubation the medium was discarded by centrifugation at 4°C and the retinas were washed twice in Tyrode's solution.

For each determination 6 retinas, resuspended in 1 ml of 10 mM Tris–HCl buffer (pH 8.0), were homogenized in a Potter-Elvehjem at 1000 rev/min (20 strokes). Portions of 50 μl of this homogenate were employed for each determination in a final volume of 250 μl. The standard assay system for thymidine kinase contained: 20 mM ATP, 40 mM Tris–HCl buffer (pH 8.0), 5 mM $MgCl_2$, 50 μM [*Me*-^{3}H]thymidine (0.5 μCi). The incubation mixture for nucleoside phosphotransferase assay contained: 5 mM dUMP, 40 mM Tris–HCl buffer (pH 8.8), 5 mM $MgCl_2$, 40 μM [*Me*-^{3}H]thymidine (0.5 μCi). Samples were incubated at 37°C for 60 min. The reaction was stopped by means of 100 μl of 10% trichloroacetic acid and the proteins removed by centrifugation. The supernatants were applied to columns (3 × 1 cm) of Dowex-1 formate. First thymidine was eluted by 26 ml of 2 N formic acid, then thymidine nucleotides were eluted with 10 ml of 1 N ammonium formate–4 N formic acid. Aliquots of 0.5 ml of this fraction were counted for radioactivity as in [4]. Activity is expressed as nmol dTMP formed/mg protein. Protein concentration was estimated as in [7] using bovine serum albumin as standard. DNA content of retinas was determined by a modification of the Burton procedure as in [8,9].

3. RESULTS

As shown in fig.1, both thymidine kinase and nucleoside phosphotransferase change in chick embryo retina during development, but in a different fashion. Thymidine kinase reaches its highest

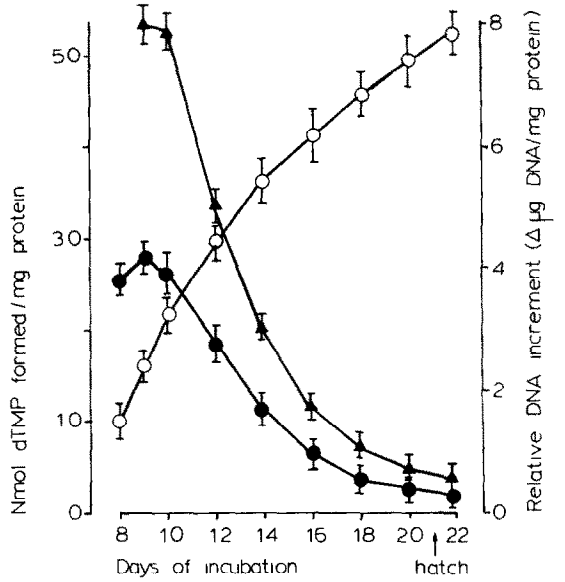

Fig.1. Thymidine kinase (●—●), nucleoside phosphotransferase (○—○) and relative DNA increment (▲—▲) in chick embryo retinas as a function of age. The activities are expressed as nmol thymidine monophosphate formed/mg protein under the conditions specified in the text. Relative DNA increment is expressed as the average daily change in the amount of DNA during a ±1 day (total 2 days) period per mg protein present that day. Each point represents the mean ± SE of 6 independent determinations.

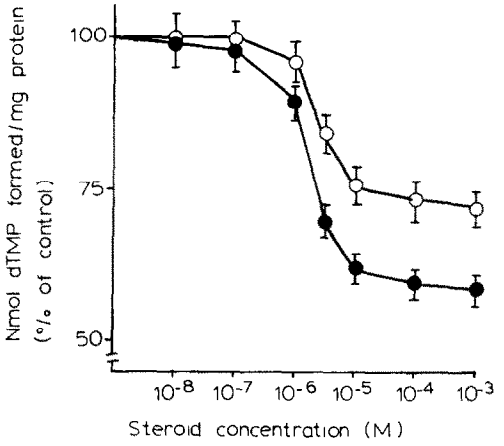

Fig.2. Dose-response effects of hydrocortisone (●—●) and cortisone (○—○) on thymidine kinase activity in isolated retinas of chicken embryo. Individual retinas of 12-day-old chick embryos were incubated at 37°C for 5 h under the conditions specified in the text in the presence or absence of steriod. Values, which are the mean ± SE of 4 separate experiments, are expressed as percentage of the activity measured from non-steriod-treated retinas.

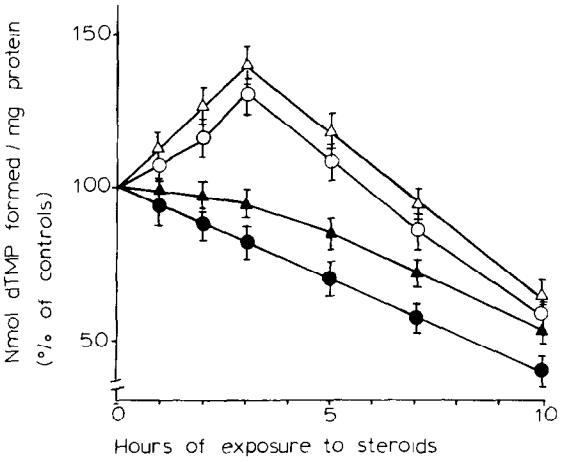

Fig.3. Thymidine kinase (closed symbols) and nucleoside phosphotransferase (open symbols) activities in isolated chick embryo retinas at various times of exposure to hydrocortisone (circles) or cortisone (triangles). Individual retinas of 12-day-old chick embryos were incubated at 37°C for various times under the conditions specified in the text in the absence or presence of 5×10^{-6} M steroid. Values, which are the mean ± SE of 4 separate experiments, are expressed as percentage of the activity measured from non-steroid-treated retinas.

values in the first phase of development; after day 10 it declines at first rapidly and then more slowly reaching the lowest value at time of hatching. The rate of DNA synthesis essentially follows this activity (fig.1). In contrast nucleoside phosphotransferase increases progressively during development.

As shown in fig.2, hydrocortisone at a concentration greater than 10^{-6} M reduces the level of thymidine kinase in isolated retinas on the 12th day. The effect increases with the time of exposure of the retinas to the steroid (fig.3). A different behaviour was observed for nucleoside phosphotransferase. Hydrocortisone at 5×10^{-6} M increases promptly this activity in the first period of incubation. After 3 h nucleoside phosphotransferase activity also declines and at 7 h it is below that of control (fig.3). Lower concentrations of hydrocortisone had no effect on this activity. Similar effects on both activities were observed with 5×10^{-6} M cortisone, although in this case the decrement of thymidine kinase was minor, irrespective of incubation time, and the increment of nucleoside phosphotransferase found in the first period of incubation was more consistent (fig.3).

The data reported in table 1 indicate that corticosterone and prednisolone also significantly reduce thymidine kinase in isolated retinas after 5 h of exposure. Among the steroids tested hydrocortisone was the most effective inhibitor. The table shows that, after 5 h of exposure, all the steroids tested increase nucleoside phosphotransferase activity, cortisone being the most effective.

Table 1

The effects of glucocorticoids on thymidine kinase and nucleoside phosphotransferase activities in isolated retinas of chicken embryo

Steroids		Thymidine kinase		Nucleoside phosphotransferase	
		nmol dTMP formed/mg protein	% inhibition	nmol dTMP formed/mg protein	% increment
None	(6)	1.70 ± 0.08		2.9 ± 0.10	
Hydrocortisone	(6)	1.20 ± 0.11**	29.4	3.19 ± 0.10*	10.0
Cortisone	(6)	1.45 ± 0.07	14.7	3.4 ± 0.12**	17.2
Corticosterone	(5)	1.35 ± 0.10*	20.6	3.2 ± 0.08*	10.3
Prednisone	(4)	1.60 ± 0.15	5.9	3.3 ± 0.10*	13.7
Prednisolone	(5)	1.40 ± 0.10*	17.6	3.0 ± 0.14	3.6

Individual retinas of 12-day-old chicken embryo were incubated at 37°C for 5 h as described in the text in the absence or presence of 5×10^{-6} M steroid. The results are expressed as mean ± SE with the number of experiments indicated in parentheses.

* $p < 0.05$ *vs* none
** $p < 0.01$ *vs* none

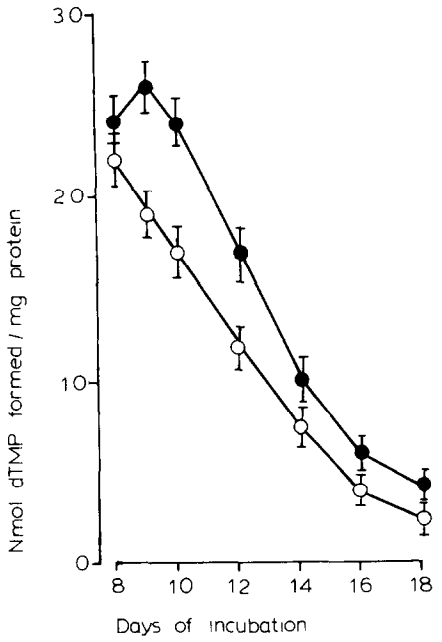

Fig.4. The effect of hydrocortisone on retinal thymidine kinase activity of chick embryo at various stages of development. Individual retinas were incubated for 5 h at 37°C under the conditions reported in the text in the presence (○—○) or absence (●—●) of 5×10^{-6} M hydrocortisone. Values, expressed as nmol d-TMP formed/mg protein, are the mean ± SE of 5 separate experiments.

A decrement of thymidine kinase activity was observed as a response to treatment with hydrocortisone in isolated retinas from 8–18-day-old chick embryos (fig.4). An inhibition of the same magnitude, about 27–30%, was observed at the various stages of development, except in retinas on the 8th day, where thymidine kinase was inhibited by only 8%.

4. DISCUSSION

Thymidine kinase and nucleoside phosphotransferase change in chick embryo retina during development in a similar fashion to that reported in chick cerebral hemispheres in [10] for the former and in [11] for the latter. Thymidine kinase is essentially correlated with the rate of DNA synthesis during the development of chick embryo cerebral hemispheres [10]. The results in fig.1 suggest a similar correlation also in chick embryo retinas. In contrast nucleoside phosphotransferase is clearly not coupled with the rate of DNA synthesis both in cerebral hemispheres [11] and in retinas.

Glucocorticoids cause two different effects on nucleoside phosphotransferase activity. The increment of activity which was observed after brief periods of exposure of retinas to hydrocortisone may not involve enzyme synthesis. It is possible that this effect is a consequence of other phenomena much as an activation of preformed enzyme or a reduction in the concentration of an inhibitor compound. Because the exposure of retinas to hydrocortisone for longer periods of time causes a decrement of activity, it is likely that other factors may be responsible for the increment of nucleoside phosphotransferase which occurs during development.

Our results indicate the glucocorticoids may be responsible for the marked fall in thymidine kinase found precociously in retinas after day 10 of chick embryo development. These data confirm that at day 10 the receptors for the steroids are already present in cells.

Piddington [12] has observed that the function of the adrenal cortex of chicken embryos increases appreciably after the 14th day of development, but that steroidogenesis is active already in adrenal glands from 12-day-old embryos. This observation supports the view that glucocorticoids can be the natural factor which decreases retinal thymidine kinase activity. Therefore, the decrement in the level of this enzyme would be a precocious effect of the glucocorticoid preceding the maturation of adrenal glands.

These observations make it of interest to ascertain whether also in vivo the retinal thymidine kinase system may be subject to control by glucocorticoids.

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