AGE-DEPENDENT STIMULATION BY ESTRADIOL-17β OF DNA POLYMERASE α IN IMMATURE RAT UTERUS

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1. Introduction

The mammalian cell has been shown to contain three classes of DNA polymerase (EC 2.7.7.7): α, β and γ [1,2]. The specific role played by these enzymes in DNA synthesis and replication is still uncertain. In general, rapidly dividing cells show elevated levels of DNA polymerase α activity as compared with non-dividing cells [3–7]. On the other hand, DNA polymerase β activity is no higher in rapidly dividing cells [3,5]. DNA polymerase γ activity [8] constituting only 1–2% of total cellular DNA polymerase activity [9], has been shown to increase in the S phase of HeLa cell growth [10].

The action of the potent estrogen estradiol-17β on the rat uterus has been extensively investigated [11,12]. The hormone causes rapid growth and proliferation of uterine cells in an age-dependent manner [13–15]. However, little is known of the mechanism whereby estrogen leads to increased DNA synthesis. This study of the effect of estradiol-17β on uterine DNA polymerases suggests that the effect of the hormone on DNA synthesis may be mediated by an increase in the activity of DNA polymerase α.

2. Materials and methods

2.1. *Preparation of enzyme extracts*

Immature rats (Wistar derived) received an intraperitoneal injection of estradiol-17β (170 ng/g body weight – ranging from 2 μg/10-day-old rat to 7.5 μg/25-day-old rat) or vehicle. Animals were killed by cervical dislocation at the time indicated. Uteri were excised, and homogenized [13] in a cold solution containing 0.25 M sucrose, 50 mM Tris–HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol (DTT). Total cell extract was prepared from the homogenate as described elsewhere [16]. Sucrose gradient centrifugation was performed using a 10–20% linear sucrose gradient containing 50 mM Tris–HCl (pH 7.5), 500 mM KCl, 5 mM $MgCl_2$, 5 mM DTT and 0.1 mg/ml bovine serum albumin (BSA). Samples of total cell extract (1–2 mg protein in 0.4 ml) were applied to 5-ml gradients. Centrifugation was at 45 000 rev./min for 15 h in an SW50.1 rotor.

2.2. *DNA polymerase assay*

DNA polymerase activity was measured both under low and high salt conditions. The low salt assay (taken as a measure of DNA polymerase α activity) contained in a final volume of 50 μl: Tris–HCl (pH 8.0) 100 mM, $MgCl_2$ 2 mM, activated calf thymus DNA 0.5 mg/ml, BSA 0.5 mg/ml, DTT 1 mM, dATP, dGTP and dCTP each at 25 μM and [^{3}H]TTP 25 μM (308 cpm/pmol). The high salt assay (taken as a measure of DNA polymerase β activity) contained, in addition to the above, 250 mM KCl. To prevent inhibition of low salt DNA polymerase activity by KCl, enzyme extract was added to give a final concentration of < 10 mM KCl. After 30-min incubation, trichloroacetic acid-insoluble material was collected on glass fibre filters (GF/C) and radioactivity measured using a liquid scintillation spectrometer. Proteins were determined by the method of Lowry et al [17].

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3. Results

The activity of uterine DNA polymerase α of the 20-day-old rat began to rise at 16 h following estrogen administration, reached a peak value of 150% at 20–36 h and returned to control levels by 48 h (fig.1). Over this period no significant variations in DNA polymerase β were observed. The age dependence of the effects of estrogen on these enzymes was investigated 24 h after hormone administration; in uteri of 10- or 15-day-old rats, no significant effect of estrogen could be observed. However, the uterus of 20-day-old and 25-day-old rats did respond to estrogen, showing a significant increase in DNA polymerase α activity (fig.2), but no marked effect on DNA polymerase β activity. Lower (but still physiologically effective) doses of estradiol-17β also elicited an increase in enzyme activity: when 50 ng of estradiol-17β was administered to 20-day-old rats the stimulation of DNA polymerase α activity was 75%

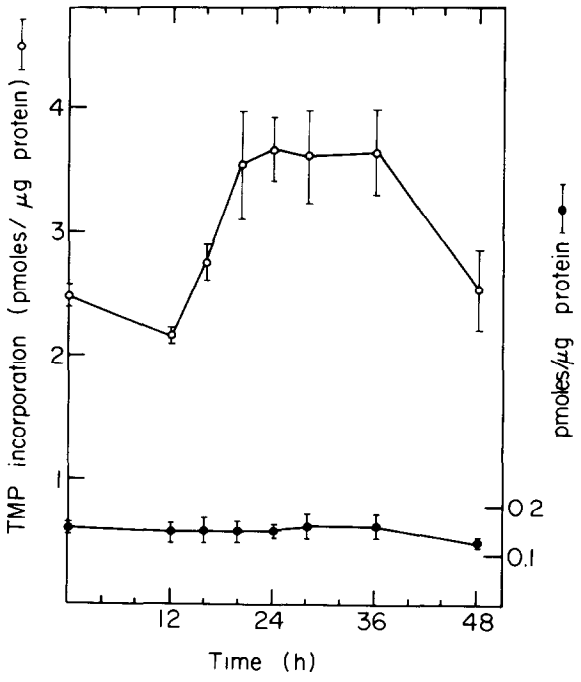

Fig.1. Time course of stimulation of uterine DNA polymerase α activity by estradiol-17β in the 20-day-old rat. Activity was measured, under low salt (○) and high salt conditions (●). Values shown are the mean ± SEM of 3–4 independent determinations on groups of 4 animals per time point.

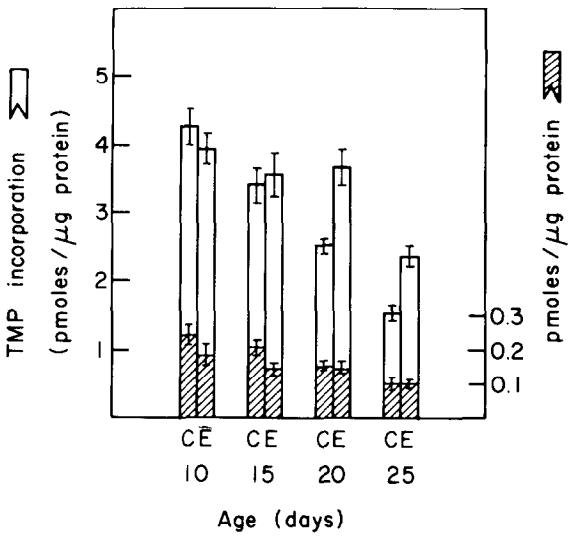

Fig.2. Age dependence of the stimulation of uterine DNA polymerase α activity by estradiol-17β at 24 h after administration. Activity was measured under low salt (open bars) and high salt conditions (hatched bars). C = untreated; E = estrogen-treated rats. Values shown are the mean ± SEM of 3–4 independent determinations on groups of 4 animals per age group.

of that observed with the 5 μg dose routinely used in this study. The hormonal specificity of the response was demonstrated by the inability of 50 ng of estradiol-17α, the biologically weakly active isomer, to cause any stimulation of DNA polymerase α.

Total cell extracts from 15- and 20-day-old rats were applied to sucrose density gradients (fig.3). Two major peaks of activity were obtained at low salt, one at 6–8 S (DNA polymerase α), the second at 3–4 S (DNA polymerase β). As in other systems, DNA polymerase α activity was abolished at high salt, whereas DNA polymerase β activity was much less affected. As found above (fig.2), no increase in activity of DNA polymerase α or β was observed in extracts of 15-day-old estrogen-treated rats. On the other hand, in extracts from estrogen-treated 20-day-old rats a significant increase in activity of DNA polymerase α but not β was observed (fig.3).

In common with the enzymes from other sources [2], rat uterine DNA polymerase α was found to be more sensitive than β to *N*-ethyl maleimide, to phosphonoacetic acid and to KCl (table 1). Both enzymes

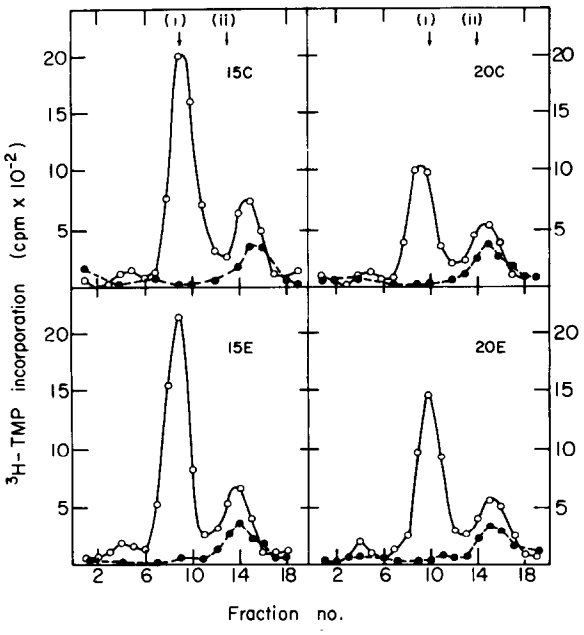

Fig.3. DNA polymerase activity in fractions of sucrose gradients of total uterine extracts from 15- and 20-day-old rats. Fractions (14 drops) were collected and 2 μl samples were assayed at low salt (○) and high salt conditions (●). C = untreated animals; E = estrogen-treated animals. The amount of protein applied to each gradient was 1.4 mg (15C), 1.3 mg (15E), 0.94 mg (20C), 0.93 mg (20E). The arrows represent the position of (i) immunoglobulin G (6.7 S) and (ii) hemoglobin (4.3 S). The direction of sedimentation was from right to left. The decrease in ratio of activity of DNA polymerase α to β following sucrose gradient centrifugation (cf. figs.2 and 3) is attributable to a partial loss (50–60%) of DNA polymerase α activity under the conditions of centrifugation (15 h at 2°C).

required added activated DNA, but only DNA polymerase α required the presence of all 4 deoxyribonucleotides for full activity [9]. Using conditions favourable for detection of DNA polymerase γ (100 mM KCl, 1 mM $MnCl_2$ and 20 μg/ml $(rA)_n$·$(dT)_{12}$ [18]) no activity was observed in crude uterine extracts nor in the fractions containing maximal activity of DNA polymerase α and β from sucrose gradients of crude uterine extracts.

4. Discussion

A good correlation exists between the kinetics of estrogen-mediated changes in DNA polymerase α activity (fig.1) and uterine DNA synthetic rates [13]. The poorer correlation between these parameters at 36 h following hormone treatment indicates that, at this time, factors other than DNA polymerase α become rate limiting for DNA synthesis. An analogous situation has been described for synchronised HeLa cell cultures [10].

It has previously been shown in our laboratory [19], that the developing rat uterus only gradually acquires full responsiveness to estrogen. Thus, the 15-day-old uterus responds to estrogen with increased overall protein and RNA synthesis but no increase in the rate of synthesis of DNA (as measured by incorporation of thymidine). On the other hand, by 20 days of age, the uterus has acquired the ability to respond with increased DNA synthesis, which is followed closely by a wave of cell division. This implies that between 15 and 20 days after birth the uterus acquires the machinery necessary for a complete response. Our present finding that the activity of uterine DNA polymerase α was stimulated by estrogen in 20- but not 15-day-old rats suggests that the absence of a full growth response to estrogen in the younger animal may be due to the inability of the hormone to provoke an increase in activity of DNA polymerase α at this age.

Table 1
Properties of separated DNA polymerase α and β

Conditions	Enzyme activity (%)	
	α	β
Complete system	100	100
+ *N*-ethyl maleimide (5 mM)	5	48
+ Phosphonoacetic acid (10 μg/ml)	42	96
+ KCl (100 mM)	31	107
+ KCl (250 mM)	< 1	46
– activated DNA	< 1	2
– (dATP, dGTP, dCTP)	49	118

Total cell extracts of uteri of 15-day-old rats were fractionated on sucrose gradients. Peak fractions of α and β activity were assayed under low salt conditions

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