0014-4754/96/080795-04$1.50 + 0.20

Effects of female sex hormones on polyamine-oxidizing enzyme activities and polyamine concentrations in immature rat uterus and liver

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Received 27 June 1995; received after revision 25 October 1995; accepted 21 December 1995

Abstract. 17β-estradiol (E_2) and progesterone (P) treatment of immature female rats (10 μg/100 g body weight) respectively resulted in 1.38-fold ($p < 0.02$) and 1.42-fold ($p < 0.02$) increase in the uterine polyamine oxidase activity, and 2.45-fold ($p < 0.001$) and 1.43-fold ($p < 0.02$) increase in the uterine diamine oxidase activity, as compared to the controls. E_2 caused a 5-fold ($p < 0.05$) and a 1.36-fold ($p < 0.05$) increase in putrescine and spermidine concentration respectively in rat uterus. Increases of 1.7-fold ($p < 0.02$) and 1.6-fold ($p < 0.05$) in putrescine and spermine concentration were determined in the P-treated uterus, as compared to the controls. The spermidine/spermine ratio, which is regarded as an index of growth rate, was higher in the E_2-treated uterus and lower in the P-treated uterus than in the control uterus. No statistically significant hormonal effects were estimated in the immature liver. The data reported suggest the possibility of an involvement of polyamine-oxidizing enzymes in the modulation of polyamine concentrations in rat uterus by the female sex hormones.
Key words. Estradiol; progesterone; polyamine oxidase; diamine oxidase; polyamines; uterus; liver.

The polyamines putrescine, spermidine and spermine are normal cellular constituents essential for cell growth and differentiation[1]. Evidence accumulated from in vivo experiments suggests that polyamines are implicated in estrogen-dependent cell growth in related target organs[2,3]. It is well known that estradiol (E_2) can modulate polyamine pools in rat uterus by stimulation of the activity of ornithine decarboxylase (ODC, EC 4.1.1.17). ODC is a key regulatory enzyme in the polyamine biosynthetic pathway, which catalyzes the formation of putrescine from L-ornithine[2,4]. There is also a report[5] showing that the activity of ODC in chick oviduct can be induced by both E_2 and progesterone (P), but the ODC induction by E_2 is higher than that caused by P. This finding is consistent with the suggestion that the rapid, manyfold induction of ODC activity by E_2 is associated with the tissue growth response, since P stimulates the synthesis of a specific protein (avidin) but does not promote oviduct growth. Apart from the tightly regulated synthetic pathway, intracellular polyamine levels are also controlled by a series of catabolic reactions. The influence of both E_2 and P on polyamine catabolism has been poorly investigated[6,7]. Oxidation represents a crucial reaction by which polyamines enter catabolic routes for functional inactivation and elimination. Polyamines and their natural N^1-acetyl derivatives are degraded by the action of two enzymes: polyamine oxidase (PAO, EC 1.5.3.3) and diamine oxidase (DAO, EC 1.4.3.6)[8,9]. PAO is involved in the polyamine interconversion pathway, in the course of which spermine and spermidine are first acetylated at N^1-position by spermidine/spermine N^1-acetyltransferase (EC 2.3.1.57). These N^1-acetylated polyamines are substrates for PAO, which splits off 3-acetamidopropanal, thereby producing spermidine and putrescine[8]. The putrescine formed can be reutilized for synthesis of the higher polyamines spermidine and spermine. The terminal oxidation of polyamines is catalyzed by DAO. This enzyme oxidizes mainly putrescine to products that are not reutilizable in the polyamine pathway[9]. We have recently demonstrated the presence of PAO activity in rat uterus[10], and the question of whether PAO activity is under control of the female sex hormones has attracted our interest.

In the present study, we investigated the effects of E_2 and P on the activities of polyamine-oxidizing enzymes PAO and DAO as well as on the intracellular polyamine levels in immature rat uterus and liver. The study was undertaken in order to evaluate the influence of female sex hormones on the processes of polyamine catabolism, and its hormonal and organ specificity.

Materials and methods

Chemicals. Chemicals used in this study were obtained from the following sources: 17β-estradiol, progesterone, N^1-acetylspermine trihydrochloride, putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, semicarbazide hydrochloride, HEPES sodium salt, MOPS sodium salt, peroxidase, bovine serum albumin, sucrose, EDTA, and ninhydrin reagent,

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from Sigma Chemicals Co. (St. Louis, MO, USA), 4-aminoantipyrine, 5-sulphosalicylic acid and propylene glycol-1,2 from Fluka Chemie AG (Buchs, Switzerland); phenol from Ferak Laborat. GMBH (Berlin, Germany).

Treatment of animals. Immature female Wistar rats, 22 days old, were used. They were given 17β-estradiol or progesterone (10 μg/100 g body weight, dissolved in propylene glycol) by intraperitoneal injection. The control rats received propylene glycol only. Animals (n = 10 for each group) were decapitated 24 h after injection and the uterus and liver were removed immediately.

Enzyme assays. For PAO activity assay the tissues were homogenized (5%, w/v) in ice-cold 0.25 M sucrose containing 10 mM HEPES (pH 7.2) and 1 mM EDTA. The homogenate was centrifuged at 3500 × g for 10 min, and the supernatant was collected and centrifuged at 20 000 × g for 20 min. The resulting pellet (a light mitochondrial fraction) was suspended in 0.25 M sucrose containing 2 mM MOPS (pH 7.2), 5 mM EDTA, and thus was used as a source for PAO activity assay. For DAO activity assay, the tissues were homogenized (20%, w/v) in 0.01 M sodium phosphate buffer (pH 7.0). The homogenate was heated at 60 °C in a waterbath for 10 min and centrifuged at 20,000 × g for 20 min. The supernatant was used as an enzyme source for DAO assay.

The PAO and DAO assay methods were based on our previously reported method[11] and that of Hayashi et al.[12] Hydrogen peroxide, formed in the amine oxidase reaction, was measured photometrically by coupling 4-aminoantipyrine with phenol in the presence of peroxidase.

The standard reaction mixture for PAO activity assay (3.0 ml final volume) contained 50 mM glycine-NaOH buffer (pH 9.5), 0.82 mM 4-aminoantipyrine, 10.6 mM phenol, 12 IU of peroxidase, 2.5 mM N^1-acetylspermine and 300 μl enzyme preparation. After incubation at 37 °C in a waterbath with shaking for 60 min, the reaction was stopped by chilling the tubes on ice. The absorbance was measured at 500 nm in a cuvette of 10 mm light path, against a blank containing all components except the substrate.

The standard reaction mixture for DAO activity assay (3.0 ml final volume), just before the photometric measurement, contained 0.1 M sodium phosphate buffer (pH 7.4), 0.82 mM 4-aminoantipyrine, 10.6 mM phenol, 12 IU peroxidase, 2.5 mM putrescine, 1.0 mM semicarbazide and 300 μl enzyme preparation. The enzyme assay was carried out as follows: the blanks (containing buffer, enzyme source and peroxidase) were preincubated with semicarbazide (a specific DAO inhibitor) at 37 °C in the waterbath for 20 min. Samples containing the same components except semicarbazide were also preincubated under the same conditions. After adding 4-aminoantipyrine, phenol and putrescine to the blanks and samples, all tubes were incubated at 37 °C in the waterbath with shaking for 60 min. The reaction was stopped by chilling the tubes on ice and semicarbazide was added to the samples. The absorbance of samples was measured at 500 nm in a cuvette of 10 mm light path, against a blank.

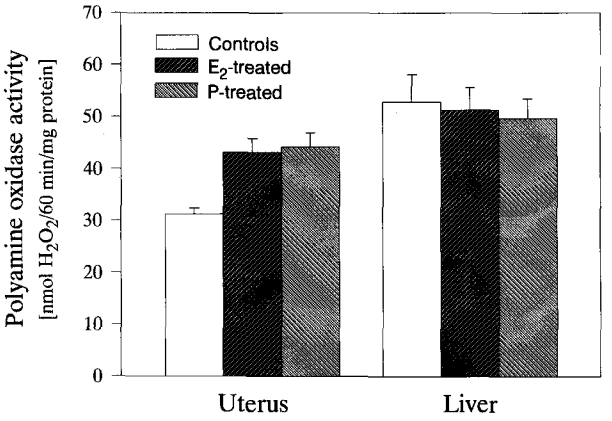

Figure 1. Effect of estradiol and progesterone on polyamine oxidase activity in rat uterus and liver. Immature rats were treated with E_2 (estradiol) or P (progesterone) as described in 'Materials and methods'. Uteri from identically treated animals were pooled into a single homogenate. The liver from each animal was separately homogenized. Values are means ± SE of 3 experiments.

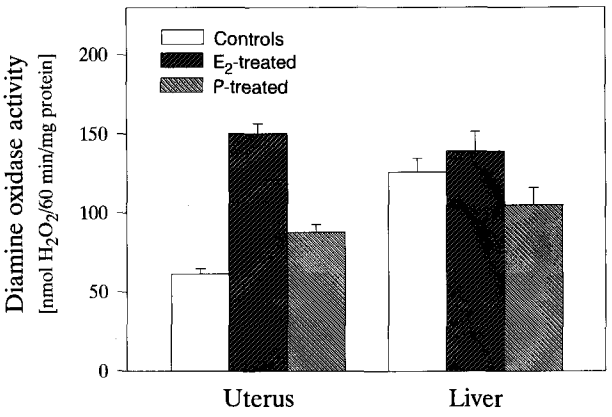

Figure 2. Effect of estradiol and progesterone on diamine oxidase activity in rat uterus and liver. Experimental details are described in figure 1. Values are means ± SE of 3 experiments.

Determination of polyamine concentrations. Tissues were homogenized in 20 volumes of 4% (0.16 M) 5-sulphosalicylic acid. Following a 1 h extraction at 0 °C, the samples were centrifuged at 8000 × g for 20 min. The supernatants were used for analysis. Polyamines were analyzed by the method of Fujita et al.[13], as described previously[14], using an amino acid analyzer 'Microtechna T-339' (Czech Republic) with photometric determination by ninhydrin reaction.

Measurement of protein content. Protein content was determined by the method of Lowry et al.[15], using bovine serum albumin as a standard.

Statistical analysis. The statistical significance of differences between two groups of data was estimated by the

Table 1. Effects of estradiol and progesterone on polyamine concentration in rat uterus and liver*.

Treatment	Polyamine concentration [nmol/g wet weight]**			
	putrescine	spermidine	spermine	spermidine/ spermine
Uterus				
controls	29.5 ± 3.5	214.4 ± 22.5	77.1 ± 17.7	2.8
E_2-treated	145.9 ± 32.5	291.1 ± 4.3	77.4 ± 20.7	3.9
P-treated	50.1 ± 3.8	237.9 ± 22.5	123.4 ± 12.8	1.9
Liver				
controls	28.0 ± 9.8	375.9 ± 57.6	204.5 ± 48.5	1.8
E_2-treated	19.9 ± 9.1	337.7 ± 58.5	185.9 ± 56.7	1.8
P-treated	24.3 ± 8.1	386.3 ± 38.1	209.1 ± 39.5	1.8

*Experimental details as described in figure 1. **Values are means ± SEM of 3 experiments.

Table 2. Effects of estradiol and progesterone on rat uterus and liver wet weight.

Treatment	Wet weight [g]*	
	uterus	liver
Controls	0.054 ± 0.004	1.789 ± 0.119
E_2-treated	0.068 ± 0.004	1.727 ± 0.098
P-treated	0.056 ± 0.007	1.943 ± 0.125

*Values are means ± SEM of 10 animals.

Student t-test. The values were considered significant when $p < 0.05$.

Results

The effects of E_2 and P on PAO activity in immature rat uterus and liver are presented in figure 1. E_2-treated rats exhibited 1.38-fold higher ($p < 0.02$) uterine PAO activity than non-treated animals. An increase of 1.42-fold ($p < 0.02$) in uterine PAO activity was found in P-treated animals as compared to the controls. No statistically significant effects of female sex hormones on hepatic PAO activity were observed.

The effects of female sex hormones on DAO activity in the organs studied are shown in figure 2. Uterine DAO activity was 2.45-fold higher ($p < 0.001$) in E_2-treated and 1.43-fold higher ($p < 0.02$) in P-treated rats than in the controls. No statistically significant effects of the female sex hormones on the activity of DAO in rat liver were determined.

Table 1 shows the effects of E_2 and P on polyamine concentrations in the organs studied. The concentrations of putrescine and spermidine in the uterus of E_2-treated rats compared with those of the control rats showed a 5-fold ($p < 0.05$) and 1.36-fold ($p < 0.05$) increase, respectively. Increases of 1.7-fold ($p < 0.02$) and 1.6-fold ($p < 0.05$) in putrescine and spermine concentrations respectively were estimated in the uterus of P-treated rats, compared with the controls. The differences observed in the hepatic polyamine concentrations between the hormone-treated and the control groups were found not to be statistically significant. The ratio of spermidine/spermine was higher in the E_2-treated uterus and lower in the P-treated uterus than in the control, but no changes in this ratio were observed in either E_2-treated or P-treated liver, as shown in table 1.

The influence of E_2 and P on the wet weight of the organs studied is shown in table 2. A 1.26-fold increase ($p < 0.05$) in the wet weight of E_2-treated uterus was the only statisticaly significant effect observed.

Discussion

The data presented show that both E_2 and P are able to stimulate polyamine-oxidizing enzyme activities in the immature rat uterus. Moreover, changes in polyamine concentrations in rat uterus in response to E_2 and P administration were observed. It is therefore possible to assume that female sex hormones can modulate polyamine pools in rat uterus by an alteration of the polyamine catabolic reactions. The effects observed suggest that the influence of the female sex hormones, especially of E_2, on the polyamine-oxidizing enzyme activities could be another way of modulating the polyamine pools, in addition to the well-known regulation of polyamine biosynthesis. Since our first aim was to describe the influence of female sex hormones on polyamine catabolism, we used a relatively high dose of estradiol, which is, however, comparable with the doses used in most of the papers concerning estrogen effects on polyamine metabolism. It would be of interest to investigate the effects of several estradiol concentrations in further experiments.

Organ specificity of the hormonal influence was demonstrated by the fact that no E_2 and P effects were observed in the immature rat liver.

The activity of PAO, responsible for the oxidative splitting of the acetyl derivatives of spermine and spermidine in tissues, was induced by both E_2 and P to an equal extent (fig. 1). Recently Hayashi et al.[12] have demonstrated a significant increase in rat liver PAO activity under various cell-proliferative conditions, suggesting the possibility of an important role of this enzyme in polyamine interconversion and putrescine formation in rapidly growing tissues. Data from the present experimental model, showing an increased uterine PAO activity in addition to the increased putrescine concentration determined, are in accordance with such a suggestion. It appears that the explanations for the increase in putrescine concentration in E_2-treated uterus, based entirely on the activation of ODC[2], might be expanded to include a role for PAO.

In several experimental models of rapid cell growth, a high ODC activity is accompanied by a high DAO activity[9,16]. The dramatic induction of ODC is followed by accumulation of its product, putrescine, which is,

however, not as striking as would be expected on the basis of the rise in ODC only. The increased DAO activity in these cases could be related to removing the excess of putrescine, which directly or through the synthesis of 'antizyme' may inhibit ODC activity[17–19]. In this way, DAO might contribute to the activation of polyamine biosynthesis and turnover. Results from the present study showed that the activity of DAO, which catalyzes putrescine degradation, was significantly stimulated by E_2 (fig. 2). However, no decrease, and even a significant increase, in the putrescine pool in E_2-treated uterus was observed (table 1). The explanation of the above findings could be related to the well-known significant stimulation of de novo putrescine biosynthesis in uterus by E_2. If we assume that the activation of uterine ODC activity by E_2 exceeds the stimulation of DAO activity, the increased putrescine concentration in uterus should not be surprising. Futhermore, the E_2 stimulation of uterine PAO activity is likely to cause an additional increase in putrescine concentration. The physiological significance of the elevated DAO activity in E_2-treated uterus might be associated with a removal of the putrescine-dependent inhibition of ODC and activation of polyamine synthesis and interconversion.

It is known that during the estrous cycle DAO activity in rat uterus is highest at proestrus and lowest at estrus, when estrogen plasma levels are, respectively, high and low[20]. The present data on the E_2 effect on uterine DAO activity in immature rats could be considered to support the finding that uterine DAO activity in the rat is a function of the circulating estrogens.

The administration of P to the immature rats resulted in an increase of uterine DAO activity (fig. 2) and putrescine concentration (table 1), but these fluctuations were slighter than those caused by E_2. It might be suggested that the possible ODC induction by P would not be so dramatic as that caused by E_2.

An involvement of new mRNA and protein synthesis in the estrogenic stimulation of uterine DAO activity has been reported[7]. It is possible that the stimulatory effect of P on DAO activity may involve such a molecular mechanism, but its confirmation requires further investigation.

It is well recognized that rapidly growing tissues frequently exhibit high putrescine concentrations and a high spermidine/spermine ratio[21], although the values of this ratio are not general indicators of growth. In the present study an increased putrescine concentration was determined in both E_2- and P-treated uteri, with a more significant increase in the E_2-treated uterus (table 1). Furthermore, a higher spermidine/spermine ratio in the E_2-treated uterus (due to an increased spermidine concentration) was observed. These results could be associated with rapid growth, which resulted in an increase in the uterine wet weight (table 2). These findings are in accordance with the well-known profound trophic effect of E_2 on uterine growth. The decrease in the spermidine/spermine ratio, which is due to an increased spermine concentration in P-treated uterus, might reflect the specificity of the P effect on the uterine growth status. The lack of fluctuation in the polyamine concentration and spermidine/spermine ratio, as well as in the wet weight in both E_2- and P-treated livers, is in accordance with the lack of trophic effect of female sex hormones on the growth processes in the liver.

Since Branham et al. have shown that E_2 is not able to affect ODC activity in rat liver[2], and our results show that E_2 does not affect the polyamine-oxidizing enzyme activities and polyamine concentrations, it could be suggested that polyamine metabolism in rat liver is not under an estrogenic control.

Acknowledgements. This work was supported by the Fund for Scientific Research from the University of Sofia 'St Kliment Ohridski', Bulgaria.

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