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Effects of female sex hormones on the activity of serum hyaluronidase

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The activities of serum hyaluronidase from female rats were measured during pregnancy. In pregnant female rats, the activity of serum hyaluronidase was found to increase initially and to fall to a minimum by the last day of the gestation period, but the activity of the enzyme increased after delivery and was similar to normals at 21 days post-partum. The activity of hyaluronidase obtained from bovine leucocytes was significantly increased when leucocytes were incubated with various concentrations of progesterone. It is suggested that female sex hormones affect lysosomal membranes making them lyse more readily and hence release hyaluronidase.

Pregnancy; Hyaluronidase; (Rat serum; Bovine leukocyte)

1. INTRODUCTION

A number of metabolic changes are observed during pregnancy which is considered to be a unique physiological condition. As pregnancy progresses, metabolically active hormones are formed and released into the maternal circulation [1]. Therefore, pregnancy is associated with a rise in the circulating levels of sex hormones [2,3].

Hyaluronidase has been demonstrated in many tissues of animals including humans [4]. The activities of serum hyaluronidase were studied among normal individuals and patients with several rheumatic diseases and it was found that 33% of the patients with active rheumatoid arthritis had serum hyaluronidase activity above the normal range [5]. However, maternal serum hyaluronidase levels have been measured during pregnancy in unselected groups of pregnant women [6,7]. It was found that there was an increase in serum hyaluronidase activity as pregnancy progressed.

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The purpose of the present study is to test the effect of female sex hormones on the activity of hyaluronidase obtained from rat serum or bovine leucocytes to provide more information on whether the increased level or activity of serum hyaluronidase is a direct effect of raised hormone levels on the lysosomes which are considered to be the source of hyaluronidase of many tissues.

2. MATERIALS AND METHODS

2.1. *Materials*

Biochemicals were purchased from Sigma (Poole, England) and used without further purification. All other chemicals were purchased from BDH (Poole, England).

2.2. *Animals*

Female Wistar rats (200–250 g) were obtained from King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Rats were maintained on a standard laboratory diet (Oxoid, modified 41B) fed ad libitum with free access to water. Animals were housed in collective cages in a light cycle and

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temperature controlled room (12 h on-off; 24 ± 1°C). Gestation was timed from the appearance of spermatozoids in vaginal smears.

2.3. *Preparation of leucocytes from bovine blood*

Fresh blood was collected directly from animals at the Jeddah slaughterhouse into bottles containing 0.2 M sodium citrate as anticoagulant. The blood was stored on ice until arrival at the laboratory and used immediately. Leucocytes were isolated from blood according to Wyss et al. [8] with slight modifications. The isolated leucocytes were either incubated with progesterone for 1 h or sonicated for 30 s.

2.4. *Separation of serum from rat blood*

Rats were anaesthetized with sagatal (0.1 ml/100 g body wt) and blood samples were collected directly from the heart with a sterile hypodermic syringe. Freshly clotted blood samples were centrifuged at 4000 × *g* for 15 min at 4°C. Serum was separated with a clean Pasteur pipette.

2.5. *Assay of hyaluronidase*

Hyaluronidase activity under optimal conditions at pH 3.9 was measured at 37°C by the colorimetric method described in [7]. One unit of enzyme activity is defined as the production of 1 μmol of *N*-acetylglucosamine/min. Immediately before assays, serum or leucocytes were diluted 1:10 (v/v) with 0.15 M NaCl.

2.6. *Expression of results*

All maximal enzyme activities are expressed as μmol/min per ml serum. The results are expressed as means ± SD and comparisons between controls and experimental data were made by Student's *t*-test.

3. RESULTS AND DISCUSSION

At day 21 of gestation the pregnant rats showed augmented body weight as compared to virgin controls (table 1). The activity of serum hyaluronidase of virgin, pregnant and post-partum female rats is listed in table 1. There was a significant increase in enzyme activity from the virgin rats to the 11–12th day of gestation. The activity of serum hyaluronidase started to fall after 12 days of pregnancy until it reached a minimum at 20–21 days of pregnancy (below the normal levels of virgin rats). The activity of the enzyme again rose significantly ($p < 0.001$) at 1–3 days post-partum. At 20–21 days post-partum enzyme activity was similar to that of virgin controls (table 1).

Table 1

Maximal activities of serum hyaluronidase of pregnant and post-partum rats

Animal status	*n*	Body wt (g)	Serum hyaluronidase activity (μmol/min per ml)
Virgin	6	239 ± 13.43	3.71 ± 0.28
Pregnant			
11–12 days	6	298 ± 7.94[b]	5.52 ± 0.34[b]
16–17 days	6	366 ± 4.79[c]	3.94 ± 0.37
20–21 days	6	420 ± 21.71[c]	2.48 ± 0.38[a]
Post-partum			
1–3 days	6	275 ± 7.29[a]	5.63 ± 0.38[c]
21 days	6	254 ± 6.29	3.65 ± 0.19

Rats were anaesthetized with sagatal, blood was collected directly from the heart with a hypodermic syringe and serum was separated. Hyaluronidase activities were determined as described in section 2. Results are presented as means ± SD, where *n* is the number of rats used. The statistical significance (Student's *t*-test) of differences between virgin rats and pregnant or post-partum rats is indicated by: [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$

The effect of inclusion of progesterone and sonication on hyaluronidase activity of leucocytes obtained from bovine blood is shown in table 2. The activity of hyaluronidase was significantly increased ($p < 0.01$ or $p < 0.001$) when 2.5 or 5 mM progesterone was incubated with leucocytes, respectively. A significant increase ($p < 0.001$) in enzyme activity was also observed when leucocytes were sonicated (table 2).

It has been reported that progesterone levels in pregnant rats increased to a maximum level at the 14th day of pregnancy and started to fall before parturition to reach a minimum in the last 2 days of pregnancy [9]. Moreover, Martin et al. [10] found that the concentration of progesterone in maternal plasma of rats decreased to low levels 1 day before birth. However, these observations

Table 2

Maximal activities of hyaluronidase in the leucocytes of bovine blood

	n	Hyaluronidase activity (μmol/min per ml)
Control	4	0.47 ± 0.04
Inclusion of 2.5 mM progesterone	4	0.62 ± 0.03[a]
Inclusion of 5 mM progesterone	4	0.75 ± 0.04[b]
Sonication of leucocytes	4	0.82 ± 0.04[b]

Leucocytes were prepared as described in section 2. Hyaluronidase activities were determined as previously described. Results are presented as means ± SD, where n is the number of samples used. The statistical significance (Student's t-test) of differences between control and experimental values is indicated by: [a] $p <$ 0.01 and [b] $p < 0.001$

would support the present results which showed that the activity of serum hyaluronidase reached a minimum in the last 2 days of pregnancy and rose after delivery, indicating that progesterone may have a direct effect on the activity of serum hyaluronidase.

The fragility of endometrial lysosomes was reported to be increased during the secretory phase of the menstrual cycle [11], and there is other evidence that progesterone may have a direct effect on lysosomal membranes, making them more permeable (unpublished). All these studies suggest that progesterone could possibly decrease the stability of the lysosomal membrane. The hormone concentrations used here were higher than the physiological levels, e.g. in blood, but if, as suggested, certain tissues can bind hormones, then during times of high hormone levels, local concentrations could approach those required in the in vitro experiments. There is now a possible explanation for the higher levels of serum hyaluronidase observed during pregnancy in humans; as the female sex hormone levels are more fragile and more liable to rupture and release, amongst other lytic enzymes, hyaluronidase.

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