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Changes in Phosphatase Activity of the Mouse Uterus during the Estrous Cycle

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發情週期에 따른 생쥐子宮의 Phosphatase 活性의 變化에 관하여

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적 요

발정주기에 따라서 생쥐자궁의 alkaline phosphatase와 transport ATPases의 활성변화를 알아보기 위하여 정량적으로 분석하였다.

발정주기의 각 시기에 있어서 이 효소활성들의 비율은 대체로 그 양상이 서로 비슷하나, 발정기의 K^+-dependent와 Na^+, K^+-activated ATPases를 제외한 다른 효소들의 활성은 다른 어떤 시기보다도 유의하게 ($p<0.025$) 높았다. 즉, K^+-dependent와 Na^+, K^+-activated ATPases의 활성은 발정간기에서 발정기에 이르는 동안 무시할 정도이고, 다만 발정후기에 약간의 활성 (0.04~0.05 μM/mg protein/hr, 총활성의 6~7%)이 나타났다.

한편, 발정기에서 Mg^{++}-dependent phosphatase, transport ATPase와 alkaline phosphatase의 활성들은 급속히 현저하게 증가하였으며 각각 0.69 (35%), 0.42 (21%), 1.58 (79%)였다. Alkaline phosphatase는 전 발정주기를 통해 0.60~1.58 (79~90%)의 활성을 보여 그 주종을 이루었다. Alkaline phosphatase의 활성중에는 Mg^{++}-dependent의 것이 총활성의 12~16%로 추정되었다.

그러므로 K^+-dependent와 Na^+, K^+-activated ATPases는 발정기 때에 자궁액의 누적을 조절하는 요인이 아니고 발정후기에서 자궁상피 속으로 내액을 재흡수하는 요인인 것으로 짐작되며, 또한 Mg^{++}-dependent phosphatase, transport ATPase 그리고 alkaline phosphatase는 생쥐의 자궁상피세포에서부터 내액을 분비하는 데에 밀접히 관련되어 있는 것으로 사려된다.

INTRODUCTION

It is well known fact that mammalian uterus shows the cyclic changes in morphology and function during the estrous cycle. Nilsson (1959) reported that the ultrastructural changes in endometrium and its gland cells were observed during the estrous cycle in the mouse. Recently, it has been demonstrated by histochemical methods that the activity and distribution pattern of alkaline phosphatase (Finn and McLarene, 1967; Aldeen, 1970; Smith, 1973) and acid phosphatase (Smith and Wilson, 1971) of the mouse uterus altered during the estrous cycle. It has been also established from the results of analysis of ions (Roblero *et al.*, 1976) and proteins (Roberts and Parker, 1972; Aitken, 1977; Fishel, 1979) including some enzymes (Breed *et al.*, 1972) that the component and/or composition of the luminal fluid changes according to the region of the genital tract and to the stage of estrous cycle even in the same animal. Furthermore, when compared with the ions and solutes of serum, it was strongly suggested that those of luminal fluid are secreted out from the uterine epithelium by active transport (Hall, 1969; Lawn, 1973) which is controlled by the activity of membrane bounded ATPase(s). Therefore, it is very interesting to study the activity of transport ATPases of the uterus in connection with the secretion and absorption of luminal fluid. So far, there are few reports on the quantitative analysis of transport ATPases of the mouse uterus. The present experiments were carried out in order to analyze quantitatively the changing pattern of the activity of transport ATPases of the mouse uterus during the estrous cycle, and discuss the results in connection with secretion and absorption of the uterus.

MATERIALS AND METHODS

Random bred A-strain female mice, aged two to three-month old, were used in these experiments. Female mice with normal estrous cycle were selected by the method of vaginal smear, checking the cyclic stages every day for one week, and divided into four groups of diestrus, proestrus, estrus and metaestrus. Each group was pooled together with five animals of the same cyclic stage. The animals were sacrificed by cervical dislocation and removed their uteri. Thereafter, the wet uteri were immediately weighed with an electric balance (Mettler, H10) and homogenized in ice-cold distilled water with a glass blender (Pyrex). The homogenate was diluted to be 1 mg uterine tissue/ml of homogenate with ice-cold distilled water.

Analysis of the enzyme activity has been done by the method based on the measurement of p-nitrophenol which is released from p-nitrophenyl phosphate

(p-NPP) as a substrate for the enzymes under the experimental condition (Ernst, 1972 a & b). Because p-NPP is hydrolyzed by alkaline phosphatase as well as transport ATPase(s), various reaction media were designed in order to identify the enzymes of the uterine tissue (Table 1).

Table 1. The component and composition of the reaction media for the tissue enzymes.

Component	Conc. (mM)	Reaction media				
		Basic	K^+-free	Mg^{++}-free	Ouabain	Cysteine
Tris-HCl	100	+	+	+	+	+
p-NPP	2.5	+	+	+	+	+
$MgCl_2$	10	+	+	−	+	+
KCl	10	+	−	+	+	+
Ouabain	10	−	−	−	+	−
Cysteine	10	−	−	−	−	+

"+" and "−" mean inclusion in and exclusion from the components of the reaction medium, respectviely.
All of the reaction media were buffered at pH 9.0.

The basic medium provided the primary reaction solution in which reaction product (p-nitrophenol) was taken as a total activity of the phosphatases in the uterine tissue. Potassium-free medium was designed to estimate K^+-dependent ATPase activity, which was represented as the figure of difference between the reaction product in basic medium and that in K^+-free medium. Magnesium-free medium was designed to estimate the activity of Mg^{++}-dependent phosphatase. The medium containing ouabain which is known as a specific inhibitor of Na^+, K^+-activated ATPase (Fujita *et al.*, 1965; Ernst, 1972 a & b) was designed to discriminate the activity of Na^+, K^+-activated ATPase from those of other enzymes. Lastly, the medium containing cysteine, which is known as a potent inhibitor of alkaline phosphatase (Gordon, 1952; Guth and Albers, 1974), was designed to discriminate the activity of alkaline phosphatase from those of transport ATPases.

One mg uterine tissue of the homogenate was simultaneously incubated in each 2 ml of the various reaction media for 60 minutes at room temperature. In order to compensate the spontaneous hydrolysis of p-NPP during the period of incubation time, the basic medium without the homogenate, as control, was simultaneously incubated with other experimental groups. At the end of incubation, the reaction was stopped by adding 0.5 ml of 37.5% trichloroacetic acid solution and mixed well for 10 minutes. The mixture was added with 2.5 ml of 1 M Tris-HCl solution for recoloration and cetrifuged at 2000 rpm for 10 minutes and discarded the resident. The amount of p-nitrophenol, which reveals the enzyme activity, was measured by reading O.D. from the supernatant at 410 mμ with a spectrophotometer (Schimatdz MSP-50). p-Nitrophenol (Sigma) solution of a serial concentration was used for

plotting the standard curve against the reaction product. The quantitative analysis of protein of the uterine tissue was done by the method of Lowry *et. al.* (1951) using bovine serum albumin (Sigma) as standard protein. The specific activity of the enzymes was represented as μM of p-nitrophenol/mg tissue protein/hour.

RESULTS

The results of these experiments were summarized in Table 2. The wet weight of the mouse uterus at the time of estrus was 106.0 mg in average, which was two to three times as heavy as those of other cyclic stages (diestrus, 41.4; proestrus, 38.1; metaestrus, 51.5).

Table 2. The activity of phosphatase of the mouse uterus during the estrous cycle in various media.

Stage	No. of animal	Wet wt./ uterus (mg)	Reaction media				
			Basic	K^+-free	Mg^{++}-free	Ouabain	Cysteine
Diestrus	20	41.4	@0.88±0.11 *(100)	0.87±0.22 (98)	0.67±0.15 (76)	0.90±0.21 (102)	0.09±0.06 (10)
Proestrus	15	38.1	0.69±0.17 (100)	0.67±0.25 (97)	0.49±0.23 (72)	0.68±0.18 (98)	0.09±0.12 (12)
Estrus	15	106.0	2.00±0.37 (100)	2.05±0.33 (103)	1.31±0.33 (65)	2.09±0.42 (105)	0.42±0.26 (21)
Metaestrus	20	51.5	0.72±0.14 (100)	0.67±0.14 (93)	0.52±0.11 (72)	0.68±0.09 (94)	0.12±0.06 (16)

@ : Mean±SE (μM p-nitrophenol/mg protein of uterine tissue/hour).
* : The percentage (%) against the reaction product in basic medium of its cyclic stage.
Significance test : Estrus stage vs the other stages, in all kinds of the enzymes analyzed. Significant at least, $p<0.025$ (Student's test).

When taken the figure of reaction product in basic medium for the total enzyme activity (100%) of its cyclic stage, the total enzyme activity at the time of estrus was 2.00 (μM/mg protein of uterine tissue/hour) in average and significantly ($p<0.025$) higher than those of other stages (diestrus, 0.88; proestrus, 0.69; metaestrus, 0.71). However, there was no significant difference in this activity between the stages of diestrus, proestrus and metaestrus. Even though the proportional (%) patterns of the enzyme activities were similar each another between the stages of estrous cycle (Fig. 1), the absolute activities of the enzymes except K^+-dependent and Na^+, K^+-activated ATPases at the time of estrus were significantly ($p<0.025$) higher than that at any other time of the estrous cycle.

That is, the reaction products in K^+-free medium and ouabain containing medium were resulted as 93~103% and 94~105%, respectively. This indicates that the activities of K^+-dependent and Na^+, K^+-activated ATPases were negligible during

the period of time from diestrus to estrus while the little activities (0.04 ~0.05, 6~7%) of these enzymes appeared at the time of metaestrus. According to the same way of estimation, the activity of Mg^{++}-dependent phosphatase was in the range of 0.20~0.69 (24~35%), since the activity of Mg^{++}-independent phosphatase was in the range of 0.49~1.31 (65~76%). At the time of estrus, the activity of Mg^{++}-dependent phosphatase was 0.69 (35%) in average and significantly ($p<0.025$) higher than that of any other cyclic stages (diestrus, 0.21, 24%; proestrus, 0.20, 28%; metaestrus, 0.20, 28%). The activity of transport ATPase, which was represented with the reaction product in the medium containing cysteine, at the time of estrus was 0.42 (21%) in average and higher than that of any other cyclic stage (diestrus, 0.09, 10%; proestrus, 0.09, 12%; and metaestrus, 0.12, 16%). It was estimated that the activity of alkaline phosphatase was predominant throughout the estrous cycle in the range of 0.60~1.58 (79~90%). The activity of this enzyme at the time of estrus was also significantly ($p<0.025$) higher than that of any other cyclic stage. It was calculated that the activity of this enzyme included the activity of Mg^{++}-dependent one in the range of 12~16% of the total enzyme activity.

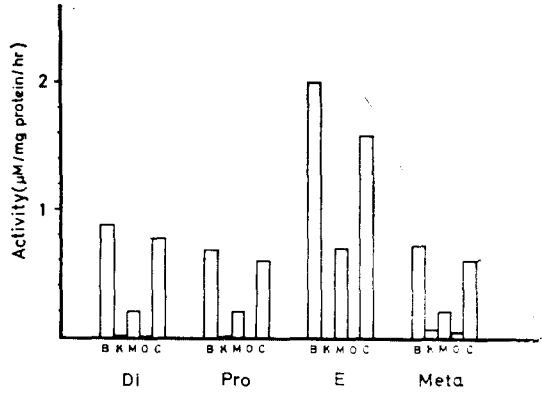

Fig. 1. The histogram of phosphatase activity of the mouse uterus during estrous cycle in the various reaction media. B, total enzyme activity; K, K^+-dependent; M, Mg^{++}-dependent; O, ouabain-sensitive (Na^+, K^+-activated); C, cysteine-insensitive (alkaline); Di, diestrus; Pro, proestrus; E, estrus; Meta, Metaestrus.

DISCUSSION

Quantitative analysis of the activities of transport ATPases as well as alkaline phosphatase of the mouse uterus during the estrous cycle was carried out using the method described by Ernst (1972 a & b), who successfully analyzed the transport ATPase activity of the avian salt gland using p-NPP as a substrate for the membrane bounded transport ATPase. The application of this method is based on several similar properties of p-NPPase to Na^+, K^+-activated ATPase (Fujita *et al.*, 1965; Albers and Koval, 1972).

When taken the figure multiplying the reaction product by the wet weight of uterus for the arbitrary integral enzyme activity of the whole uterus, although the wet weight of uterus was not exactly proportional but parallel to the amount of

protein in the uterus, the integral enzyme activity at the time of estrus was rapidly and tremendously increased in all kind of enzymes except K^+-dependent and Na^+, K^+-activated ATPase. These results are coincident with the findings of the cell proliferation (Finn and Martin, 1973), the rapid synthesis of enzymes (Wilson, 1969) and the fluid accumulation (Martin *et al.*, 1970). Recently, it was suggested that the fluid accumulation in the uterine lumen might be induced by active transport of the epithelium (Lawn, 1973). This suggestion is supported by the reports that the uterine fluid is insisted to be the secretion of the uterine epithelial cells rather than a simple oedema (Ringler, 1961), and that the uterine fluid contains some proteins different from those in the plasma (Murray *et al.*, 1972; Gore-Langton *et al.*, 1976; Aitken, 1977).

In the present experiments, there was no increase in K^+-dependent and Na^+, K^+-activated ATPases during the period of time from diestrus to estrus while these enzymes showed a little activity (6~7%) of the total enzyme activity at the time of metaestrus. These results imply that these enzymes are not the main factor to control the fluid accumulation in the mouse uterus and presumably related with the reabsorption of the luminal fluid into the uterine epithelium at the time of metaestrus. On the other hand, Mg^{++}-dependent phosphatase, transport ATPase (cysteine-insensitive phosphatase) and alkaline phosphatase showed the peaks of their activities at the time of estrus. This indicates that the secretion of uterine fluid in the mouse must be closely and directly and/or indirectly related with these enzymes. However, there is no direct evidence for the correlationship between these enzymes and the secretion and reabsorption of luminal fluid in the uterus. Further studies of identification and ultrastructural localization of these enzymes in connection with the correlationship are in progress.

SUMMARY

Quantitative analysis of the activities of transport ATPases as well as alkaline phosphatase of the mouse uterus was carried out during the estrous cycle. Even though the proportional patterns of the enzyme activities were similar each another between the stages of estrous cycle, the absolute activities of the enzymes except K^+-dependent and Na^+, K^+-activated ATPases at the time of estrus were significantly ($p<0.025$) higher than that at any other time of the estrous cycle.

That is, the activities of K^+-dependent and Na^+, K^+-activated ATPases were negligible during the period of time from diestrus to estrus while the little activities (0.04~0.05 μM/mg protein/hr in average, 6~7% of the total enzyme activity) of these enzymes appeared at the time of metaestrus. On the other hand, at the time of estrus, the activities of Mg^{++}-dependent phosphatase, transport ATPase

and alkaline phosphatase were rapidly and tremendously increased to be 0.69 (35 %), 0.42 (21%) and 1.58 (79%), respectively. The activity of alkaline phosphatase was in the range of 0.60~1.58 (79~90%) and predominant throughout the estrous cycle. The activity of Mg^{++}-dependent alkaline phosphatase was estimated as 12~16% of the total enzyme activity.

Therefore, it is assumed likely that K^+-dependent and Na^+, K^+-activated ATPases are not the main factors to control the fluid accumulation at the time of estrus, but may be the factors to reabsorb the luminal fluid into the uterine epithelium at the time of metaestrus, and that Mg^{++}-dependent phosphatase, transport ATPase and alkaline phosphatase must be closely involved in the secretion of luminal fluid from the epithelial cells of the mouse uterus.

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