ON THE SIMILARITY BETWEEN RIBONUCLEASE INHIBITOR AND β-ESTRADIOL RECEPTOR PROTEIN

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Summary. Normal rat uterus tissue contains no detectable free RNase inhibitor. In ovariectomized animals free RNase activity in homogenate of uterus declines and RNase inhibition occurs, but in quantity the inhibition is only 15% of that found in 20-23 day old immature rats. The similarities between β-estradiol receptor protein and RNase inhibitor are discussed and it is suggested that cytosol β-estradiol receptor protein may be the RNase inhibitor of uterus tissue.

The function of RNase inhibitor and its mode of interaction with RNase are still unknown although it appears likely that the inhibitor may be an important controlling element in RNA metabolism and protein synthesis (1). It is well established that the integrity of polysomes is dependent on the presence of RNase inhibitor in the high speed supernatant fractions obtained from mammalian tissues (2-4). In a number of systems RNase inhibitor has been found to increase when there is an increase in cell growth and proliferation (5-7) while with conditions in which growth is inhibited or protein synthesis is decreased, RNase inhibitor activity is decreased (8-12).

It appeared that a useful system to study the functions of RNase inhibitor would be the ovariectomized and immature rat injected with 17 β-estradiol. The mechanisms of action of this hormone in promoting RNA and protein synthesis have been intensively studied (13) but little has been done with the RNase-RNase inhibitor system (14,15).

Methods. Mature 30 weeks old Charles River female rats were used as controls. Two groups of experimental animals were used. The first was immature females 20 to 23 days old. The second group was 30 week old females ovariectomized 4 to 6 weeks before the experiment. Ovariectomized rats were injected with 1.6 μg 17 β-estradiol (Sigma) every 12 hr for 4 to 6 days or with one 100 μg dose of

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Nafoxidine hydrochloride (16) (Upjohn-11,100 A). These compounds were dissolved for injection in steroid suspending vehicle (Armour Pharmaceutical Co.,).

The animals were anesthetized with ether, uteri were stripped of fat, weighed and each 50 mg net weight was homogenized with 1 ml 0.05 M Davies buffer (17) with 1 mM dithiothreitol and 1 mM EDTA, in a glass homogenizer at 0-4°. The homogenate was utilized immediately or a high speed supernatant fraction was prepared by centrifugation at 105,000 x g for 1 hr. For each sample free RNase (pH 7.4; pH 8), RNase :inhibitor complex and free inhibitor were determined. Activity of RNase was assayed in 0.05 M Davies buffer by the method of Ambellan and Hollander (18), the inhibitor:RNase complex was determined by addition of excess p-chloromercuribenzene sulfonic acid (pCMS) (10^{-3}M) to the assay system; this destroys inhibitor in the complex and releases the bound RNase. RNase inhibition was determined in the presence of 7.5 mM EDTA. From 1 to 50 µl of 5% uterus homogenate was used with 7.5 x 10^{-4} µg of pancreatic RNase.

Results and discussion. A number of points may be made from the data in Table I. First the absence of free RNase inhibitor from mature rat uterus is

Table 1. Changes of uterus RNase activity and its inhibition of bovine pancreatic RNase

Assay	Immature	Ovariectomized	Mature (control)	Ovariectomized injected for 4-6 days before assay	
				17β-estradiol	Nafoxidine
Free RNase	0	0	0.675	0.560	0.960
Bovine pancreatic RNase inhibition	48%	8%	0*	0*	0*
RNase;RNase inhibitor complex	0.650	0.170	0.160	0.160	0.410
Uterus wet weight in mg	25 ± 5	70 ± 10	430 ± 50	320 ± 50	150 ± 50

All results are the average of at least 10 animals. Data are expressed: Δ260nm/mg tissue/hr/37°. RNase inhibitor activity is expressed in terms of inhibition of 7.5 x 10^{-4} µg of bovine pancreatic RNase (EC 2.7.7.16)/mg of uterus tissue.
*In the presence of as much as 2.5 mg tissue. Free RNase is largely in the sediment and is measured at pH 8.

surprising since all other mammalian tissues examined thus far have some (1). Although uterus from ovariectomized rats seems to have some free inhibitor and immature uterus more, there is no certainty that this inhibition is indeed from RNase inhibitor. Thus, Jensen et al. (2) observed that β-estradiol receptor protein in the presence of hormone combined with RNase but he dismissed this as simply the non-specific reaction of the acidic receptor protein with the basic protein of RNase. We do not yet know if the combination of receptor protein with RNase inhibits the enzyme but are studying this point.

It is an interesting coincidence that the quantity of receptor protein in immature uterus is 75% higher than can be found in uterus from ovariectomized rats (21,22). The same relation was observed for RNase inhibition (Table I). In addition, RNase inhibitor and β-estradiol receptor protein have many properties in common. These are summarized in Table II.

The big differences in free RNase activity of uterus between immature or ovariectomized rats and mature animals are remarkable and may play some role in the control of RNA metabolism. The types and distribution of RNase among the cell particulates is unknown.

Also noteworthy is the observation that immature rats have 4 times the level of RNase released from a complex by pCMS compared to ovariectomized animals (Table I).

Free RNase and RNase inhibition activities were also determined in uterus of ovariectomized rats every hour after a single injection of 1.6 μg of 17 β-estradiol. Twelve hours after injection no RNase activity was present and RNase inhibition was high. If injections were continued at 12 hour intervals the increase in free RNase activity appeared at 48 hours, the same time that a

Table II. Similar properties of RNase inhibitor and β-estradiol receptor protein*

RNase inhibitor	β-estradiol receptor protein
Acidic protein	Acidic protein
M.W. 50-60,000	M.W. 60,000
Both proteins chromatograph in identically the same position on DEAE cellulose	
Free-SH required for activity	Free-SH required for activity
Destroyed by -SH reactants	Destroyed by -SH reactants
Combines with RNase	Combines with RNase

*Data for β-estradiol receptor protein was taken from Jensen and DeSombre (21) and Jensen et al. (22).

decrease of RNase inhibition was detected. Between 4 and 6 days after beginning injections, levels of RNase and RNase inhibition were the same as in the control group of mature rats.

This agrees with data of Teng and Hamilton (19) who found in uterus of ovariectomized rats, 50-60% higher amino acid incorporation in vitro than in vivo. In vitro incorporation decreased during prolonged 17 β-estradiol administration, and approached at 72 hr the lowest activity observed in normal animals, at the time we observed the highest activity of free RNase and the absence of RNase inhibition.

According to our results the mechanism which regulates the levels of RNase and RNase inhibition activities in uterus may be under the control of 17 β-estradiol. Use of Nafoxidine (16) in place of 17 β-estradiol gives the same effect, i.e. a decrease of RNase inhibition and appearance of free RNase (Tab. I).

It is possible that 17 β-estradiol receptor protein is the RNase inhibitor of uterus tissue and perhaps its activity can be modified by the presence of hormone. These and other interesting possibilities are being studied as well as the types and distribution of RNase activity in uterus.

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