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ACID RIBONUCLEASE ACTIVATION IN THE CHICK OVIDUCT BY TISSUE DAMAGE, ACTINOMYCIN D AND PROGESTERONE

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SUMMARY

A low acid RNase activity was found in the nuclear and cytoplasmic fraction of the immature chick oviduct. The most rapid induction of cytoplasmic RNase was caused by oviductal ligation at 2 h, whereas actinomycin D and progesterone effects on RNase appeared at 10 and 24 h after administration. Nuclear acid RNase activity was much lower and increased concomitantly with the cytoplasmic RNase except for the progesterone-treated oviducts. This indicates that nuclear acid RNase may be a cytoplasmic contamination. The lysosomal origin of this acid RNase and its possible role in avidin induction is discussed.

INTRODUCTION

Avidin is a biotin-binding secretory protein of the chick oviduct which can be induced by progesterone treatment of the immature chick [1]. Our previous studies indicate that avidin can also be induced by damage to the oviductal tissue (ligation) and actinomycin D [2], progesterone not being necessary in the latter case. Tissue damage causes destruction of lysosomes and release of the lysosomal hydrolases, e.g. acid ribonuclease (RNase), acid phosphatase and β-glucuronidase [3]. It would appear that similar lysosomal activation also occurs in the chick oviduct after ligation. On the other hand, there is evidence for steroid-enhanced liberation of lysosomal hydrolases and transfer of lysosomes in specific hormone target cells [4–6]. According to Szego[5, 6] lysosomes may mediate specific steroid-induced responses in target organs. Determination of acid RNase has been used as an assay for lysosomal activation [6]. We therefore investigated the effects of ligation, actinomycin D and progesterone on lysosomes of the chick oviduct by measuring acid RNase activity.

EXPERIMENTAL

Animals. One-day-old Leghorn chicks (strains Ti 53 and Ti 453, Turun Muna Hatchery, Turku, Finland) were injected subcutaneously with 0.5 mg of diethylstilboestrol (DES, Merck) in 0.05 ml of propylen glycol daily for 9 days in order to stimulate oviduct growth. The chicks were used for experiments one day after the last DES injection. The animals weighed then 100 ± 30 g. A dose of 5 mg of progesterone (Merck) in 0.2 ml of propylen glycol per 100 g body weight was injected subcutaneously and 50 μg of actinomycin D (Sigma) in 0.3 ml of saline per 100 g body weight was given intraperitoneally. The oviducts were ligated as previously described [7]. Chicks were killed 2, 10 or 24 h after the treatments.

Tissue samples. The oviducts were dissected free of membranes and frozen immediately with dry ice and stored at -20°C until the assay. The tissues were homogenized in a Waring blender (13,500 rev./min) for 30 s at $+4$°C with 2.5 ml of a buffer/g of tissue (0.25 M sucrose, 4 mM $MgCl_2$, 10 mM KCl, 1 mM methanethiol in 20 mM Tris–HCl, pH 7.5). The homogenate was centrifuged at $+4$°C 10,000 rev./min for 15 min. Supernatant was used for cytoplasmic RNase assay. The nuclear pellet was suspended in the homogenization buffer and filtered through cheese cloth. The suspension was layered on an equal volume of 0.32 M sucrose in the homogenization buffer and centrifuged at 10,000 rev./min for 10 min. The nuclear fraction was suspended in 0.2 M sodium acetate buffer, pH 4.5 and used for nuclear RNase assay.

Acid RNase assay. Acid RNase activity was assayed according to the method of Takahashi[8]. The reaction mixture contained a 0.1 ml sample of the supernatant (cytoplasmic) or nuclear pellet, 0.25 ml of 0.2 M sodium acetate buffer, pH 4.5, 0.1 ml 0.02 M EDTA aq. solution, 0.3 ml distilled water and 0.25 ml RNA (1 mg/ml, freshly prepared before use, Sigma). The incubation time was 15 min at 37°C after addition of RNA solution. The incubation was stopped with 0.25 ml of 0.75% uranyl acetate in 25% perchloric acid. The reaction mixture was centrifuged at 2200 rev./min for 10 min, supernatant was diluted 1:10 with distilled water and the optical density at 260 nm was read with a Hitachi spectrophotometer. The blank was made from every sample by adding uranyl acetate immediately. The results are expressed

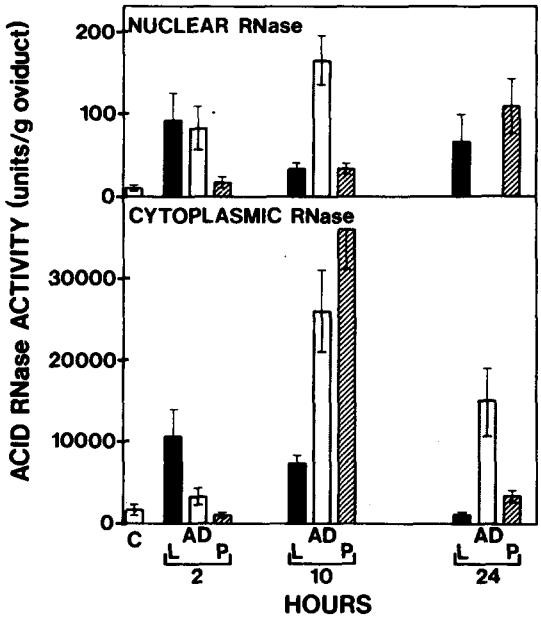

Fig. 1. Activity of cytoplasmic and nuclear acid RNase in the immature chick oviduct at time indicated after ligation (L) of the oviduct, actinomycin D (AD) and progesterone (P) treatment. Control chicks (C) received only diethylstilboestrol. Each point represents samples of 6–10 chicks, mean ± S.E.M.

as unit equivalents of pancreatic RNase standard (40 units/mg, Boehringer) per g of the oviduct wet wt.

RESULTS

Cytoplasmic acid RNase (Fig. 1)

Control oviducts show low acid RNase activity. The earliest activation of RNase was found after ligation. Here the RNase activity was still high at 10 h and decreased at 24 h. Both actinomycin D and progesterone caused the highest RNase activity at 10 h after administration. Acid RNase activity was high at 24 h after actinomycin D only.

Nuclear acid RNase (Fig. 1)

The activation of nuclear RNase occurred concomitantly with the cytoplasmic except for progesterone-treated chicks, which showed the highest activity 24 h after progesterone injection.

DISCUSSION

Our results show a 5–15-fold increase in cytoplasmic acid RNase activity after oviductal ligation, progesterone or actinomycin D administration. This is in agreement with earlier reports indicating that cell injury [3] and steroids [4–6] can activate RNase. Our results agree with the finding of Means and O'Malley[9] who described no change in chick oviductal RNase activity by progesterone at 24 h after the administration. However, we find a transient increase of the acid RNase activity 10 h after progesterone. Furthermore, our data indicate that also nuclear acid RNase is activated. Nuclear RNase activation in the chick oviduct has been recently shown to occur also after estrogen administration [10]. However, nuclear activities are always so low that they might be due to a minute cytoplasmic contamination which is likely in the nuclear isolation method used by us. On the other hand, Szego[5, 6] has shown that estrogen causes a penetration of lysosomal acid RNase II into the nucleoplasm of the uteri and preputial glands of ovariectomized rats. In addition, it has been reported that progesterone has the capacity to disrupt isolated lysosomes of liver, spleen and polymorphonuclear leukocytes [11]. This means that sex steroids may control the migration and liberation of lysosomal enzymes into the target cell nuclei. It has been suggested [10, 12] that the steroid-induced accumulation of mRNAs coding for egg white proteins in chick oviduct may be mediated by an activation of nuclear ribonucleases.

In this study we have not assayed specific acid RNase species; the acid RNase activity reported here may involve several RNases. However, this activity most likely reflects acid RNases, since we are using very low pH; 4.5, for the assay. Some acid RNases, for example acid RNase II, are known to be of lysosomal origin [6]. We therefore can assume that the detected increases in RNase activity reflect lysosomal disruption.

Actinomycin D administered intraperitoneally has been reported to cause mobilization of leukocytes and subsequent release of histamine and lysosomal enzymes into the peritoneal cavity of the rat [3]. In the peritoneal cavity of the chick actinomycin also caused severe inflammation. Thus actinomycin D induces inflammation which in turn, causes disruption of lysosomes and release of acid RNase.

Elo [2, unpublished data] has reported that avidin synthesis of the oviduct after ligation begins at 6 h, after progesterone at 10 h and after actinomycin D at 16 h. Our present data demonstrate that acid RNase is activated earlier after ligation than after progesterone and actinomycin D. Induction of avidin by ligation is located close to the site of damage [7]. Tissue damage is known to cause breaking of lysosomal membranes and release of hydrolytic enzymes [3]. It is likely that local tissue damage causes the most rapid injury to lysosomes and activation of hydrolases. Hence ligation of the oviduct causes a rapid induction of avidin. It can thus be concluded that lysosomal activation always precedes avidin synthesis induced by the respective treatments, which would agree with Szego's statement that lysosomes may mediate hormone action [4–6]. Furthermore, our results clarify why a specific progesterone response, avidin synthesis, can be induced by tissue damage. It remains unsolved, however, whether or not this lysosomal activation is a part of the mechanism of avidin induction in the chick oviduct.

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