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Gonadotropin Regulation of Rat Ovarian Lysosomes: Existence of a Hormone Specific Dual Control Mechanism

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Gonadotropic hormones PMSG (15 IU/rat), FSH (3 μg/rat), LH (9 μg/rat) and hCG (3 μg/rat) were shown to decrease the free cytosolic lysosomal enzymes during the acute phase of hormone action in rat ovaries. When isolated cells from such rats were analyzed for the cathepsin-D activity, the granulosa cells of the ovary showed a reduction in the free as well as in the total lysosomal enzyme activities in response to FSH/PMSG; the stromal and thecal compartment of the ovary showed a reduction only in the free activity in response to hCG/PMSG. The results suggest the presence of two distinct, target cell specific, mechanisms by which the lysosmal activity of the ovary is regulated by gonadotropins.

KEY WORDS: gonadotropins; PMSG; FSH; LH; hCG; lysosomes; cathepsin-D.

ABBREVIATIONS: PMSG, pregnant mare serum gonadotropin; FSH, follicle stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; GC, granulosa cells; S/T, stromal and thecal cells.

INTRODUCTION

Ovarian follicular atresia is a cellular degenerative process, the biochemical mechanism of which is poorly understood. Since cellular degenerative processes are always associated with the increase in lysosomal hydrolases (1), we described a model system to study atresia wherein the changes in lysosomal enzymes are quantitated following priming of immature rats with PMSG (2, 3). In this paper, we report our data on the modulation of the rat ovarian lysosomal stability by gonadotropins and the existence of two different control mechanisms in the ovarian lysosomal machinery.

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MATERIALS AND METHODS

Animals

Twenty one day old Wistar rats bred in the I.I.Sc. colony were used. The colony was maintained on a light dark schedule of 14:10 hrs. The animals were fed a standard pellet diet (Hindustan Lever, India) with water *ad libitum.*

Hormones and Chemicals

Highly purified ovine follicle stimulating hormone (oFSH-G4211B), pregnant mare serum gonadotropin (PMSG-PM68; 1750 IU/mg) and ovine luteinizing hormone (oLH-G3-281GB) were gifts from Professor H. Papkoff, San Francisco, CA, USA. Human chorionic gonadotropin (hCG-CR121; 12000 IU/mg), ovine follicle stimulating hormone (NIH-FSH-S13) and PMSG (NIH-PMSG; 2000 IU/mg) were gifts from Hormone Distribution Program, NIH, Bethesda, USA. The PMSG antiserum used (CH 55-Hyde) was raised in a chimpanzee, and characterized for its specificity.

The following enzyme substrates and chemicals were obtained from Sigma Chemical Company, St Louis, Missouri, USA: bovine hemoglobin, bovine serum albumin, and p-nitrophenyl phosphate. All other reagents were of AnalaR grade and they were from BDH Chemicals, India.

Determination of Lysosomal "Total" and "Free" Activity

The animals were killed by cervical dislocation; the ovaries were trimmed off the surrounding tissue in a Petri dish containing Kreb's Ringer Bicarbonate buffer, pH 7.2 containing 1 mg/ml glucose (KRBG). Ovaries of several rats were pooled for each group and 4% homogenate was prepared in 0.25 M sucrose containing 0.02 M KCl and 0.001 M EDTA using a Potter Elvehjem homogenizer (Arthur H. Thomas) according to the methods of Dimino and Reece (4). An aliquot of this homogenate designated as "total" activity was saved and stored at −70°C for enzyme analysis. The remaining aliquot was centrifuged at $35,000 \times g$ for 2 h in a Sorvall RC 2B centrifuge using a SM 24 rotor. The supernatant representing the "free" activity of lysosomal hydrolases was stored at −70°C till enzyme activity determination. The "total" activity denotes the specific activity of the lysosomal hydrolases present in the cytosol plus that present as the lysosomal "bound" form in the cell. The "free" activity denotes the specific activity of the enzyme which exists freely in the cytosol, presumably, released from the lysosomes.

Granulosa Cell Isolation

The ovarian cells were isolated by the methods described by Vidyashankar and Moudgal (5). The cells were homogenized and processed for enzyme assays as described earlier (2).

Determination of Enzyme Activities

The lysosomal marker enzymes, acid phophatase (EC 3.1.3.2) and cathepsin D (EC 3.4.4.23) were assayed as described in our earlier report (2). The activity of each enzyme was measured in the linear portion of the curve with respect to time and protein concentration. All the enzyme assays were done in triplicate with appropriate enzyme blanks. Acid phosphatase activity was expressed as nmoles of *p*-nitrophenol released per mg protein per min; cathepsin-D activity was expressed as nmoles of tyrosine equivalents released per mg protein per min. Protein was determined by the method of Lowry *et al.* (6).

Statistics

Each value represents the mean ± S.D. of 6 animals from a typical experiment. The data were analyzed statistically by Student's "t" test or by analysis of variance followed by Duncan's multiple range test. Only P values below 0.05 were considered significant.

RESULTS AND DISCUSSION

Using lysosomal enzymes as markers to study the ovarian cellular degenerative process called atresia, we established that the lysosomal enzymes of ovarian granulosa cells were under the control of gonadotropic hormone (2, 3). In the present study, to distinguish the different hormonal regulatory mechanisms which control the ovarian lysosomes, both the "total" (lysosome bound plus cytosolic) and the "free" (cytosolic) activities of lysosomal enzyme, cathepsin-D, were monitored. When 15 IU PMSG was administered, 24 h later the "total" activity did not show any change in the specific activity, whereas the "free" activity showed a reduction of 40% and 20% in the specific activities of lysosomal markers cathepsin-D and acid phosphatase respectively (Table 1).

A dose response curve showed that the maximal effect (reduction in the "free" activity) was at a dose of 45 IU and the minimal effective dose was 15 IU (data not shown). A time course study of this response showed that the reduction in the "free" activities of cathepsin-D and acid phosphatase activities could be seen from 12 h onwards after a single injection of 15 IU of PMSG, as shown in Fig. 1. The maximal reduction in the "free" activity was observed from 18 h to 48 h after PMSG. At 72 h, the "free" activity showed an increase and this was more significant in the case of cathepsin-D. At all these time points the "total" activity did not show any significant change. The "free" activity exhibited two distinct phases. An initial decrease in the activities of acid phosphatase and cathepsin-D,—suggesting lysosomal stabilization,—was followed 48 h later by an increase in their activities (Fig. 1).

The increase in the enzyme activities observed at later time points indicated that the stabilization of lysosomes upon PMSG treatment could be a function of the availability of gonadotropin support. Probably, at the later time points beyond 48 h, due to a significant reduction in PMSG levels (as a consequence of

Table 1. Effect of treatment with 15 IU PMSG on ovarian lysosomal enzymes

Acid Phosphatase activity			
Treatment	Non-fractionated homogenate (Total activity)	35,000 × *g* supernatant (Free activity)	% Change
None	38.0 ± 6	34.0 ± 2	24%
PMSG	42.0 ± 4	25.7 ± 2*	
Cathepsin-D activity			
Treatment	Non-fractionated homogenate (Total activity)	35,000 × *g* supernatant (Free activity)	% Change
None	65.8 ± 10.9	17.0 ± 2.0	36%
PMSG	69.2 ± 11.8	11.0 ± 1.0*	

Enzyme assays were performed following the procedures given in "Materials and Methods". The values represent mean ± S.D. of 6 animals from a typical experiment. Acid phosphatase activity is expressed as nmoles of *p*-nitrophenol released per mg protein per min; cathepsin-D activity is expressed as nmoles of tyrosine equivalents released per mg protein per min. *Values are significantly different at $p < 0.01$ from control values.

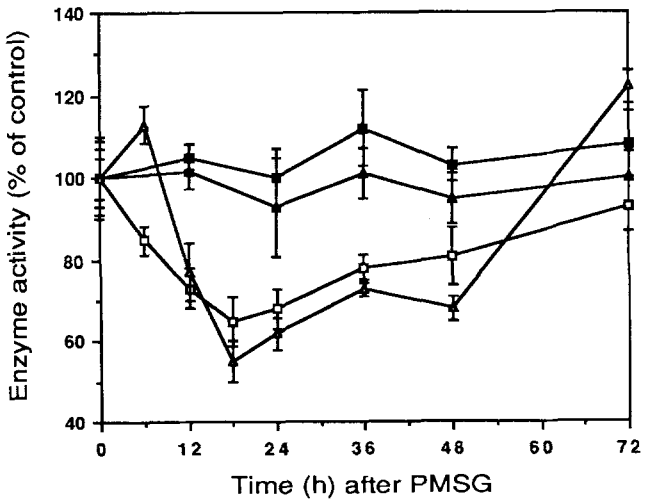

Fig. 1. Effect of 15 IU PMSG on the activities of ovarian lysosomal hydrolases with time. The specific activities were plotted as the percent of the respective control values. (■), acid phosphatase total activity; (□), acid phosphatase free activity; (▲), cathepsin-D total activity; (△), cathepsin-D free activity. 18, 24, and 48 h values are different at $p < 0.01$ from that 0 h control.

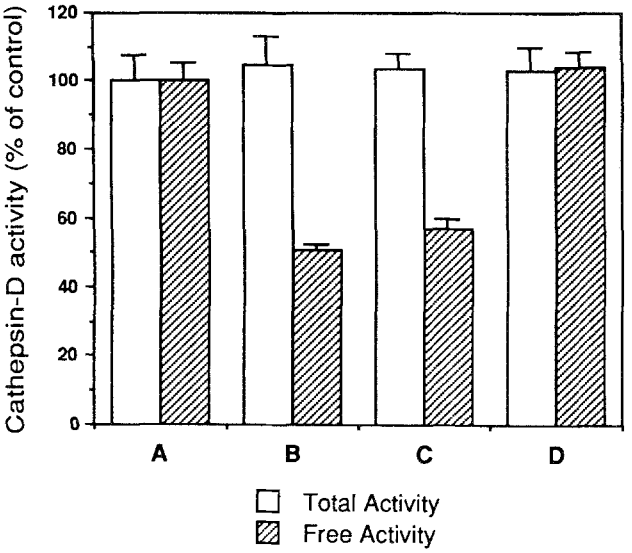

Fig. 2. Effect of PMSG a/s on the PMSG induced reduction in cathepsin-D activity. A: 0 h control; B: 24 h PMSG; C: 48 h PMSG; D: 48 h PMSG + PMSG a/s at 24 h after PMSG. The free activity value of PMSG + PMSG a/s group is significantly different ($p < 0.01$) from 24 h PMSG and 48 h PMSG groups.

metabolic clearance of PMSG), the labilization of lysosomes might have occurred resulting in an increase in the "free" activity. This is substantiated by our observation that an injection of PMSG a/s at 24 h after PMSG could accelerate this process and prepone the onset of the increase in cathepsin-D activity (Fig. 2). This result also suggested that the lysosomal enzyme response was a true PMSG effect.

When the specificity of different gonadotropins such as FSH, LH, and hCG were checked for their lysosomal stabilization activity (by way of measuring the ovarian cathepsin-D "free" activity), all of them showed equivalent ability to reduce cathepsin-D "free" activity (Fig. 3). Since the ovary is comprised of different target cells sensitive to either FSH or LH like gonadotropins, this observation is not surprising. The observed loss in specificity could be due to FSH acting on the granulosa cells, whereas LH acts on the stromal and thecal cells of the ovary producing the stabilization of lysosomes in these cells. In fact, that this is the case is proven by the experiment in which the different compartments of the ovary were analyzed for "free" and "total" activity (Fig. 4). As expected, FSH reduced the "free" activity of granulosa cells while hCG reduced the "free" activity of the stromal and thecal cells. PMSG, being a molecule with both FSH-like and LH-like activities, reduced the "free" activities of both the ovarian cell types. Most interestingly, both FSH and possibly the FSH component of the PMSG molecule reduced the "total" activity of the granulosa cell cathepsin-D. In contrast, both hCG and PMSG could effect the reduction only in the "free" and not in the "total" activity of the stromal and thecal cell cathepsin-D.

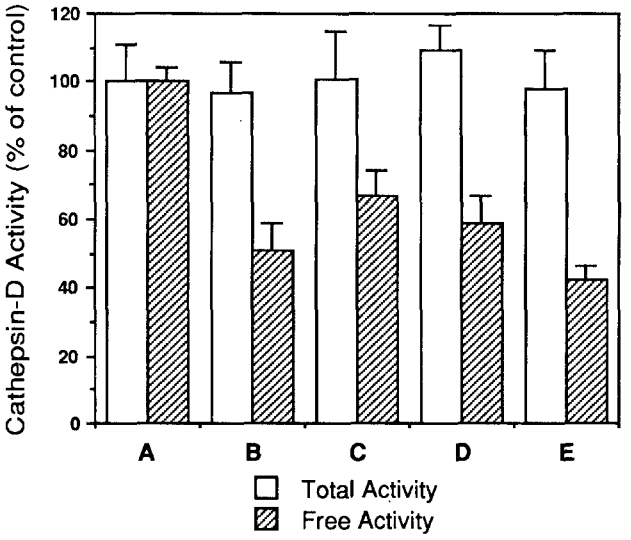

Fig. 3. Effect of different gonadotropins on the ovarian cathepsin-D activity. A: saline control; B; 15 IU of PMSG; C: 3 μg of oFSH; D: 9 μg of oLH; E: 3 μg of hCG. The free activity value of hormone treated groups are significantly different ($p < 0.01$) from saline control group.

Dimino and Reece (4) observed a similar reduction in the "free" activities of ovarian lysosomal enzymes upon treatment with PMSG for 54 h. However, they were not able to demonstrate the hormonal or cellular specificity in this process. By using isolated cellular compartments of the ovary we could clearly demonstrate the hormonal specificity of this process.

Lysosomal enzyme activation has been demonstrated to occur by two

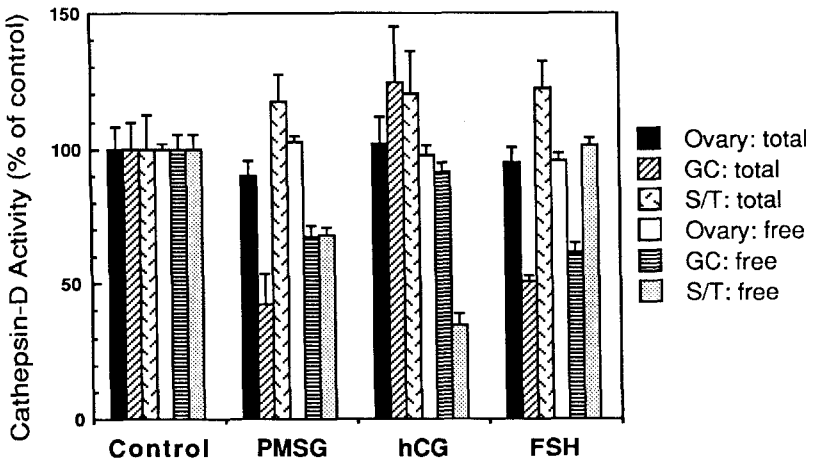

Fig. 4. Effect of gonadotropins on the total and free activities of cathepsin-D in isolated ovarian compartments. 15 IU PMSG, 3 μg hCG, and 3 μg FSH were injected to the rats. The reduced values in hormone treated groups are significantly different ($p < 0.01$) from the respective saline control groups.

different mechanisms (10–14). Mobilizing the lytic enzymes from "lysosomal-bound" pool to "free" pool, thus increasing the free activity, has been accounted in the mechanism of action of insulin (7) and steroid hormones (8). In contrast, an increase in the "total" activity of lysosomal enzymes in the cell (presumably by an increased synthesis of these enzymes) was observed during cellular degenerative processes such as uterine endometrial regression (9), mullerian duct involution (10), and mammary gland involution (11, 12). It has been known from the histological studies that during atresia the active degeneration is restricted mostly to the granulosa cells (13, 14). In this context, the reduction in the granulosa cell "total" activity (Table 1; Fig. 4) seems to be a physiologically significant mechanism by which FSH prevents granulosa cell degeneration to promote follicular growth. The stromal and thecal cells which do not undergo degeneration during the cyclical event of atresia, show hormone specific reduction, only in the "free" activity.

Our results indicate that the ovarian lysosomes are under a dual control mechanism by gonadotropic hormones: an acute control mechanism in which a specific gondotropin acts on its target cell, stabilizes the lysosomes, and reduces the release of hydrolases into the "free" cytosolic pool; a secondary and chronic mechanism, in which a gonadotropin reduces the "total" activity itself, either by reducing the synthesis of hydrolases or by synthesizing some novel inhibitors of lysosomal hydrolases. While the former control mechanism operates in granulosa, stromal and thecal cells, the latter control mechanism is present only in the granulosa cells under the control of FSH and FSH like hormones.

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