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Autoradiographic analysis of FSH binding during follicular atresia

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Summary. Binding of FSH to ovarian cells was studied in PMSG primed immature Swiss mice. 48 h after PMSG treatment, FSH-binding was higher in the periphery than in the cumulus cells of the antral follicles. Binding of FSH to granulosa cells of normal follicles was observed to be specific, 48 h after PMSG injection. No localization in the atretic follicles could be seen by autoradiography 72 h after priming.

Pregnant mares' serum gonadotropin (PMSG) is known to induce follicular maturation in rodents. Human chorionic gonadotropin (HCG) injected 48 h later can stimulate these follicles to ovulate, but in the absence of HCG these follicles do not ovulate and undergo degeneration[2,3]. Based on this observation, a model has been developed[3] by which 90% atretic follicles could be obtained 72 h after PMSG injection. In the present study, quantitative analysis of follicle stimulating hormone (FSH) binding sites during atresia were studied, using this model.

Materials and methods. Immature Swiss mice, weighing about 8–10 g, housed in controlled environment (24 °C, 60% humidity) were injected s.c. with 5 IU of PMSG (1790 IU/mg) at 16.00 h on day 21 of age and were divided into 4 groups of 5 animals each. Mice were autopsied 48 h or 72 h after PMSG injection. 2 h before autopsy, groups I (48 h-PMSG) and II (72 h-PMSG) received 5 µg per 100 g b.wt of labeled ovine FSH (L.E. Reichert, Jr), and group III (48 h-PMSG) received 0.0597 mCi/ml (volume was adjusted on the basis of radioactive counts), administration was by i.v. injection through the tail vein. Specific activity of the labeled FSH was 69 µCi/µg, iodination being performed by the Chloramine-T method[4]. To a 48 h-PMSG injected group IV the unlabeled FSH (NIH-FSH-S 11, 0.5 mg/100 g b.wt) was administered i.v. 1 h prior to the labeled material.

The animals were sacrificed, and the ovaries were removed, fixed in Bouin's fluid, embedded in paraffin and sectioned. The sections were washed, air-dried, dipped in NTB-3 emulsion and exposed at 4 °C. Three weeks later these sections were developed in Kodak-D-19 and stained with hematoxylin and eosin. For a quantitative estimation of FSH binding, grain counts were made on stained sections. The number of grains per cell of 4 antral follicles in each ovary was counted. Grain counts within the emulsion (background) adjacent to ovarian sections were also made. FSH binding was expressed as the difference between total grain counts over the granulosa and the background counts within the emulsion.

Results. Ovary 48 h after PMSG: The distribution of reduced silver grains, corresponding to bound 125_I-FSH, in the mouse ovary 48 h after PMSG in group I, was very selectively localized in the granulosa cells of antral follicles (fig. 1). The density of the grains was highest in the outer peripheral cells and lowest in the inner cumulus (table). The theca and interstitial tissue did not show localization of grains. The quantitative analysis of grain distribution in the various ovarian compartments demonstrated that the grain density in the granulosa cells was about 3–5 times higher than in the theca and interstitial compartments, in which it corresponded closely to the nonspecific value (table).

Ovary 72 h after PMSG: The binding of 125_I-FSH to granulosa cells was low in the antral follicles of mouse ovary, 72 h after PMSG injection in group II. The distribution of the silver grains was similar in the granulosa and theca cells, and was not specific (fig. 2). Quantitative analysis of the grain distribution over the various ovarian compartments demonstrated that none of the cell types showed more than 2 grains per cell (table).

When an excess of unlabeled ovine FSH (100 times more) was injected 1 h before the 125_I-FSH in group IV no specific uptake was observed in any of the cell types. Injection of labeled (free) iodide in group III did not give rise to localized silver grains in the ovary either.

Discussion. Exclusive binding of 125_I-FSH to the granulosa cells of antral ovarian follicles of immature mice 48 h after priming with PMSG, has been demonstrated in this study. Specificity of the binding of FSH to granulosa cells of antral follicles was further confirmed by its inhibition with excess of unlabeled FSH in the competitive binding test.

Distribution of radioactivity in antral mouse ovarian follicles 2 h after labeled FSH and iodide

Tissue	No. of reduced grains/cell body (mean ± SE)		
	Labeled FSH 5 µg		Unlabeled FSH 0.5 mg + labeled FSH 5 µg
	48 h	72 h	
Theca and interstitial	1.45 ± 0.24^a	0.88 ± 0.09	0.02 ± 0.01
Granulosa central (cumulus)	5.30 ± 0.42^b	1.22 ± 0.27^c	0.04 ± 0.02
Granulosa peripheral	6.65 ± 0.68^b	1.17 ± 0.20^c	0.09 ± 0.01

[b] compared to a $p < 0.01$; [c] compared to b $p < 0.001$.

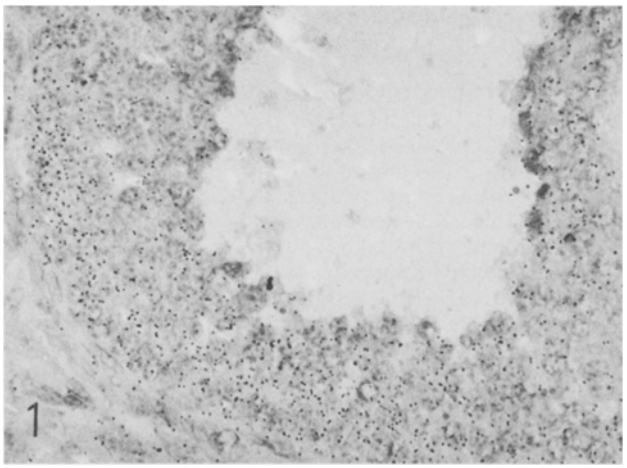

Figure 1. Autoradiograph of a section of a mouse ovary, 48 h after PMSG injection, showing radioactivity from incorporated 125_I-FSH in the peripheral granulosa and cumulus cells. Note negligible number of grains in the theca and interstitium.

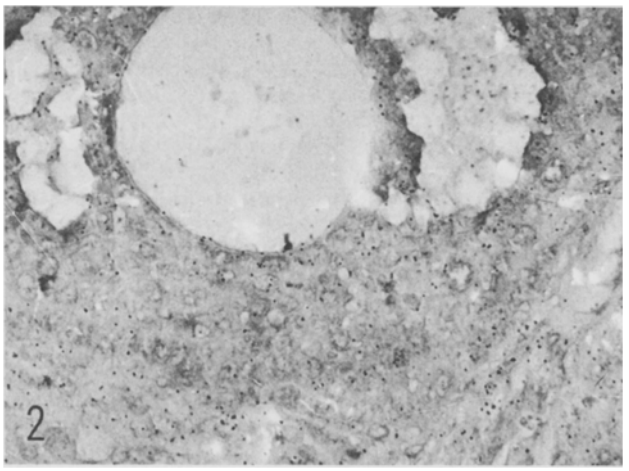

Figure 2. Autoradiograph of a section of a mouse ovary, 72 h after PMSG injection, showing radioactivity from incorporated 125_I-FSH. Dispersed sliver grains are seen all over the follicle.

The remaining cell types of the ovarian compartment did not show any uptake of labeled FSH. These results are in agreement with earlier reports[5-7].

Recently we were able to demonstrate that, morphologically, 10% of the antral follicles were atretic 48 h after PMSG injection and 90% by 72 h after PMSG treatment. Thus the decreased binding of FSH to granulosa cells, 72 h after PMSG, is presumably due to degenerative changes, possibly reducing the number of FSH-receptors in the granulosa cells of atretic follicles.

The distribution of silver grains in the peripheral granulosa cells situated near the basement membrane was higher than in the cumulus cells loated centrally in the 48 h group. This differential uptake has been reported earlier with labeled luteinizing hormone (LH[8,9]).

McNatty[10] has shown that FSH concentration in the fluid of atretic follicles is significantly reduced compared to that in normal antral follicles. The reduced FSH-binding to granulosa cells of atretic follicles, observed by us, could also be due to decreased availability of FSH in the follicular fluid due to a change in the permeability of the basement membrane. Recently, Farookhi[11] postulated that the basement membrane changes its permeability during atresia of the follicle. However, he presumes that this change in membrane permeability permits even large molecules, such as globulins (antibodies to granulosa cells) to enter the follicle and probably destroy it.

It is therefore necessary to elucidate further whether the changes in the binding of FSH to granulosa cells during follicular atresia are due to a decrease in 1. the number of FSH receptors on the granulosa cells 2. in the availability of FSH due to changes in the permeability of the basement membrane and/or 3. passage of antibodies to granulosa cells through the basement membrane, causing degenerative processes in these cells.

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Norepinephrine level in the hypothalamus of the genetically hypertensive mouse[1]

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Summary. The norepinephrine content of the hypothalamus of young mice with high blood pressure was statistically lower than that of mice with low blood pressure. The difference was not evident in older mice from these same strains. No differences in dopamine content were found suggesting a genetic difference in the activity of the converting enzyme.

There are presently a number of potential animal models for human hypertension[2,3]. The cause of the hypertension in these models is still unknown and there is still some question as to whether or not any of these models will be a suitable paradigm for human hypertension. As determinants of the elevated blood pressure in animal models are