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The role of follitropin and lutropin on the ovarian function in rats

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Abstract. Adult cycling female rats were treated with antisera to highly purified human follitropin and lutropin for eight days. The effect of this treatment on the *in vitro* steroidogenic response of the ovarian cells isolated from these rats to follitropin and lutropin has been investigated. Neutralisation of follitropin did not have significant effect on steroid production in response to lutropin. However, neutralisation of lutropin resulted in a very significant inhibition of response to both follitropin and lutropin.

Keyboards. Follitropin; lutropin; radioimmunoassay; rat; steroidogenesis

Introduction

It is well established that lutropin (LH) stimulates steroidogenesis under *in vivo* and *in vitro* conditions in a variety of species (Savard *et al.,* 1965; Armstrong, 1968; Eaton and Hilliard, 1971; Carlson *et al.,* 1971). However, recent studies (Tsafriri *et al.,* 1976; Grimek *et al.,* 1976; Dorrington and Armstrong, 1975; Armstrong and Papkoff, 1976) using highly purified preparations of ovine follitropin (FSH) have shown that FSH by itself can induce ovulation in rats, stimulate follicular adenylate cyclase and steroidogenesis under *in vivo* and *in vitro* conditions. Jagannadha Rao *et al.* (1974) have shown in a detailed study, using antiserum to LH and FSH antiserum freed of LH antibodies, that FSH has no significant role in induction of ovulation in both rats and hamsters. However, recent studies of Eppig (1979) based on the production of plasminogen activator by granulosa cells, prior to plasmin acting to weaken the follicular wall and hence ovulation, suggests that as far this criterion is concerned, ovulation is more sensitive to FSH than LH. In the present communication the relative role of FSH and LH in stimulation of production of progesterone and estradiol in rat ovarian cells after neutralisation of endogenous FSH or LH by specific antisera to human FSH and LH has been studied.

Materials and methods

Hormones

Highly purified human pituitary FSH and LH were prepared and characterized according to published procedure (Sairam *et al.,* 1978). Rat LH and FSH were obtained from the Hormone Distribution Officer, NIAMDD, Bethesda, Maryland, USA. The biological activity of FSH was monitored using a specific *in vitro* assay in

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Abbreviations: LH, lutropin; FSH, follitropin; RIA, radioimmunoassay; A/S, antiserum.

this laboratory (Jagannadha Rao and Ramachandran, 1975). LH activity was tested in the *in vitro* Leydig cell assay described earlier (Ramachandran and Sairam, 1975).

Immunisation

Antisera to human pituitary FSH and LH were raised in New Zealand white male rabbits according to the procedure of Vaitukaitis *et al.* (1971). The antisera were characterised by agar gel diffusion and quantitative precipitin tests. The ability of the antisera to neutralise the biological activities of rat FSH and LH was ascertained by testing the capacity of the antisera to inhibit the response to rat FSH and LH in the *in vitro* bioassays mentioned above.

Animals and other procedures

Sprague-Dawley female rats (160-180 *g*) were obtained from Simonsen Laboratories, Gilroy, CA, USA. Rats were used after observing three consecutive estrous cycles. To groups of five rats, each 0.5 ml of normal rabbit serum or antiserum to FSH or LH was administered for a period of eight days by subcutaneous route. Twenty-four hours after the last injection, rats were anaesthetised by nembutal, blood collected by cardiac puncture, serum separated and stored at —20°C until further processing.

Preparation of ovarian cells

The ovaries and uteri were dissected free of connective tissue and weighed in a torsion balance. From each group the ovaries were pooled, minced and taken in 30 ml of Krebs Ringer bicarbonate buffer pH 7.4 containing 2 mg bovine serum albumin, 1 mg glucose and 0.01 mg Lima Bean Trypsin Inhibitor per ml (incubation buffer). After one wash with the buffer, the minced tissue was digested in the same buffer with 5 mg collagenase (Worthington) per ml (10 ml) for 1 h at 37° C with gentle shaking. After digestion, the suspension was allowed to settle and the supernatant removed and discarded. Thirty ml of fresh incubation buffer was added to each tube and the suspension was dispersed by draining in and out of a plastic syringe with a tygon tubing at the end. The suspension was allowed to settle and the supernatant removed and filtered through two layers of cheese cloth. The process was repeated three times and filtered supernatants pooled and centrifuged gently at room temperature for 15 min at 500 g. The cell pellet was washed twice with the incubation buffer and resuspended in a known volume of the incubation buffer. A known aliquot of the suspension was diluted with sterile 0.9% saline and the cell number was determined in a coulter counter.

Incubation

From each group, cells ranging from 4×10^5 to 9×10^5/tube were incubated in a plastic tube (Falcon) in a total volume of 0.5 ml with or without FSH or LH in an atmosphere of 95% O_2 and 5% CO_2 for 2 h at 37°C. At the end of incubation the tubes were stored at –20°C until further processing.

Steroid estimation

Progesterone and estradiol –17β were estimated by radioimmunoassays standardised in this laboratory. Progesterone and estradiol –17 β bovine serum albumin conjugates were prepared according to the procedure of Erlanger *et al.* (1975). Antibodies were raised in male rabbits and characterised by checking the cross

reactivity with related steroids, namely pregnenolone, testosterone, dehydro-epi-androsterone, aldosterone and corticosterone; it was found to be less than 0.001%. Serum was thawed and extracted with 3 volumes of diethyl ether and ether layer concentrated and reconstituted in 0.01 M phosphate buffer. Recovery was monitored by addition of [^{3} H]-steroids (2000 cpm) and was found to be greater than 80%. Values reported are uncorrected for recovery. For cell suspension, after thawing 0.5 ml of phosphate buffer was added, mixed well and 0.1 ml taken for radioimmuno-assay (RIA).

Results

Administration of A/S to human FSH or LH for a period of 8 days showed differential effects on estrous cycle. With LH antiserum treatment, the animals showed an estrous-diestrous smear, the FSH-antiserum treated animals showed normal smear pattern for the first cycle and then showed a continuous atypical estrous; there was no significant decrease in ovarian and uterine weights (table 1). However, it can be

Table 1. Effect of antiserum to human follitropin and lutropin on ovarian and uterine wt and serum progesterone and estradiol –17β

		Wt. of ovaries (mg)	Wt. of uterus (mg)	Serum	
				Progesterone ng/ml	Estradiol pg/ml
A	NRS*	86.7±2.1	442.6±4.8	12.5 ±2.1	33.2±2.5
B	FSH A/S treated	80.6±4.5	448.0±6.2	6.2 ±1.0	21.1±1.03
C	LH A/S treated	77.6±1.2	303.0±11.2	1.96±0.5	10.8±1.50

All values are Mean ±S. E. of 5 observations.
Injections were given by subcutaneous route for a period of 4 days.

		'P'values
Ovaries	A - B	> 2.0
	A-C	< 0.025
Uterus	A-B	- N.S.
	A-C	< 0.005
Progesterone	A-B	< 0.025
	A-C	< 0.001
Estradiol	A-B	< 0.005
	A-C	< 0.001

* Normal rabbit serum

seen that neutralisation of LH by its antiserum was effective in decreasing the ovarian weights. The effects of depriving the endogenous gonadotropins were much more pronounced in the serum levels of progesterone and estradiol. Administration of FSH antiserum resulted in 50% and 37% decrease in the progesterone and estradiol –17 β levels, respectively. Neutralisation of LH caused a drastic decrease in the serum progesterone level and nearly 70% decrease in the estradiol level.

The affects of *in vivo* neutralisation of FSH and LH on *in vitro* steroidogenic response of ovarian cells to exogenously added FSH and LH are presented in table 2. It can be seen that in the control group, FSH caused a significant stimulation of

Table 2. Effect of *in vivo* antiserum treatment of follitropin and lutropin on the response of rat ovarian cells to exogenous follitropin and lutropin.

Hormone	Progesterone pg/10^5 cells			Estradiol -17β pg/10^6 cells		
	NRS*	FSH A/S	LH A/S	LH A/S	NRS*	FSH A/S
	NRS*	FSH A/S	LH A/S	NRS*	FSH A/S	LH A/S
None	44.0± 1.0	27.7±3.1	16.1±0.9	7.1± 2.3	9.0± 4.0	0.25±0.1
FSH	56.0± 0.8	35.9±0.2	23.2±1.4	14.0± 1.0	18.2± 0.4	0.5 ±0.1
LH	327.5±13.5	283.8±9.0	126.4±2.4	27.5±14	17.9±14	0.9 ±0.5

Amount of FSH or LH used, 1 ng
All values are mean ± S.E. of triplicate determinations
* NRS Norma rabbit serum
Progesterone and estradiol –17 β determined by RIA (see text for details).

progesterone production. However, the stimulation was much more with the addition of LH. Neutralisation of FSH resulted in a significant decrease in basal production of progesterone as well as the response to both FSH and LH. As can be expected, the response to FSH in FSH A/S treated group is significantly decreased. There is a decrease in response to LH in the FSH A/S treated group. However, it is of interest that, in the LH A/S treated group, not only the basal production of progesterone is reduced but the response to both FSH and LH is significantly reduced.

The general picture for estradiol is similar to that of progesterone as far as the groups treated with normal rabbit serum or FSH A/S are concerned. However, neutralisation of LH resulted in a very drastic decrease in the basal production and in the response of both FSH and LH. The decrease is estradiol production is much more than in the case of progesterone.

Discussion

The involvement of FSH in induction of ovulation and stimulation of steroidogenesis has been the subject of intense investigation (Nuti *et al.,* 1974; Jagannadha Rao *et al.,* 1974; Schwartz *et al.,* 1975). Thus far, all the reports concerned with the action of FSH on ovulation and steroidogenesis express some concern as to the specificity of the effects observed. However, recent studies have indicated that FSH itself has some intrinsic steroidogenesis activity in the complete absence of LH. The results of the present study using highly purified human FSH and LH and their antisera also supported such a conclusion. However, this activity is greatly reduced with prior treatment of the animal with LH A/S indicating that LH has a very significant role in the regulation of steroidogenesis. It is also possible that the decreased response of cells to FSH *in vitro* after neutralisation of LH *in vivo* may also be due to decreased FSH receptor content, which may in turn be due to decreased production of estrogen which facilitates induction of FSH receptor (Ireland *et al.,* 1978). It can be seen that quantitatively the cells produce much more progesterone than estradiol and deprival

of LH resulted in a very significant decrease in estradiol production in response to either FSH or LH. In view of the fact that estrodiol is way down in the steroidogenic pathway, this observation suggests that LH exerts its action at an earlier step and thus controls estradiol production by regulating the supply of precursor.Studies of Sheela Rani and Moudgal (1978) have shown that the block of estrogen synthesis in the cycling hamsters by LH A/S treatment is at the level of androgen synthesis. They observed that the inhibitory effect could be reversed by supplementing with testosterone under *in vitro* conditions. However, the present results show that in the FSH A/S treated group, there is no significant decrease in the estrogen production in response to FSH. This appears to contradict the observation of Sheela Rani and Moudgal (1978) who demonstrated that FSH is necessary for aromatisation of testosterone. It should be pointed out that in the present study no attempt has been made to isolate the granulosa cells or luteal cell and to sacrifice all the animals on the same day of the cycle. In view of this, the cell population includes luteal cells which could have contributed to the increased estrogen which also partly explains the lack of decrease in the weight of uterus in the FSH A/S treated group (B-table 1). Both progesterone and estrogen play an important role in the physiology of reproduction in the female and by regulating the precursor supply LH plays an important role in steroidogenesis. This partly explains the reasons for the significant and drastic effects observed following LH deprival compared to FSH deprival.

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