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THE RÔLE OF FSH AND LH IN THE INITIATION OF OVULATION IN RATS AND HAMSTERS: A STUDY USING RABBIT ANTISERA TO OVINE FSH AND LH

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Summary. The relative rôles of FSH and LH in ovulation induction in immature and adult cycling rats and hamsters have been evaluated. Both heterologous purified pituitary hormones and homologous crude pituitary extracts have been used as ovulatory stimuli in immature animals primed with PMSG. Well-characterized FSH and LH antisera have been used in the above model systems to achieve specific neutralization of FSH and LH. The present study revealed that LH is the physiological trigger needed for induction of ovulation in both rats and hamsters and FSH cannot, by itself, induce ovulation in the total absence of LH.

INTRODUCTION

Although several investigations in the past have shown the ability of LH to induce ovulation, the term 'ovulation inducing hormone(s)' continue to be used suggesting that ovulation-induction could be due to more than one hormonal stimulus. The reason for this thinking is the recent evidence which has accumulated in favour of FSH also being an ovulation inducer. This includes (a) the appearance of a concomitant FSH and LH release just before ovulation (McClintock & Schwartz, 1968; Midgley & Jaffe, 1968; Daane & Parlow, 1971), (b) the ability of highly purified FSH (not more than 1% LH contamination) to induce ovulation in hypophysectomized rats primed with PMSG (Lostroh & Johnson, 1966), (c) the ability of exogenous 'cleansed' FSH (treated with chymotrypsin or urea—Harrington, Bex, Elton & Roach (1970), or with LH antiserum—H. Lipner, N. R. Moudgal, G. J. Macdonald, S. Y. Ying & R. O. Greep, unpublished results) to induce ovulation either in intact rats treated with chlorpromazine or in hypophysectomized rats, and

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(d) the ability of general purpose gonadotrophin antiserum, reduced after absorption with NIH ovine FSH (Goldman & Mahesh, 1969), to block ovulation in hamsters.

Since some of the conclusions from the above studies were questionable (e.g. (a) absorption of FSH antibodies, from an antiserum containing antibodies to both FSH and LH, by LH, and (b) making the tacit assumption that, in intact animals treated with chlorpromazine, there is a complete shut-off of both tonic and surge LH release), it was considered essential to reinvestigate the rôle of FSH *per se* in the ovulation process. In the present study, the ovulation-inducing ability of FSH in the absence of LH and vice versa has been tested. A preliminary account, using rats (Moudgal, Jagannadha Rao, Madhwa Raj & Maneckjee, 1970) and hamsters (Jagannadha Rao, Madhwa Raj & Moudgal, 1971), has been reported earlier.

MATERIALS AND METHODS

Animals

The animals used were derived from the Indian Institute of Science colony. Immature albino rats on the 22nd day of age and golden hamsters on the 28th day of age received 20 i.u. PMSG (Ayerst) at 10.00 hours and 56 hr later a dose of ovulating hormone, either in the form of purified ovine FSH or LH or as rat or hamster pituitary extract, was injected. Adult rats and hamsters which exhibited a minimum of three regular cycles were also used in these experiments. They received the appropriate antiserum injection at 10.00 hours on the day of pro-oestrus. Autopsies were performed 18 to 24 hr after the administration of ovulatory hormone or antiserum. At autopsy, oviducts were dissected out, gently compressed between two glass slides and examined for ova under a microscope (×40). Relevant groups were subjected to statistical analysis and probability was calculated by Student's *t* test.

Hormones

The hormone preparations, NIH-FSH-S7 and S8, NIH-LH-S14, and PMSG were used. Highly purified preparations of ovine LH (1×2·5 NIH-LH-S1) and ovine FSH (1×45 NIH-FSH-S1) were used in the iodination and radiolabelled hormone-binding studies. The purified LH preparation was also used as an ovulation inducer.

Antisera

Potent antisera to NIH ovine FSH and ovine LH (Hormone Research Laboratory preparations) were raised in rabbits and characterized according to the methods described earlier (Moudgal & Li, 1961; Jagannadha Rao & Moudgal, 1970; Madhwa Raj & Moudgal, 1970). The method of characterization of FSH antiserum was further refined and the details are given below.

Antiserum raised against NIH ovine FSH preparations was characterized by initial absorption with normal sheep serum and placental extract (see Jagannadha Rao & Moudgal, 1970, for details). Antibodies to LH were specifically removed by a new method standardized in our laboratory. This involves incubating FSH antiserum (1 ml) with LH-coated tanned formalized red blood

cells (0·5 ml 1% red blood cells coated with 0·165% NIH ovine LH) as a solid immunosorbent. After incubation (accompanied by mild stirring using a magnetic stirrer) at room temperature for 1 hr, the immunosorbent was removed by centrifugation and the supernatant antiserum was checked for the presence of LH antibody. The treatment with the immunosorbent was repeated if the absorption was found to be incomplete.

The ability of the antiserum to bind and neutralize LH was tested by using ^{125}I-labelled LH in binding studies (Madhwa Raj & Moudgal, 1970) and in the OAAD test of Parlow (1961) as modified by Sakiz & Guillemin (1963). The binding study involved the incubation of 10 μl undiluted antiserum with ^{125}I-labelled hormones for 24 hr at 37°C. The antigen–antibody complex was then precipitated by adding goat antibody to rabbit γ-globulin. The incubation was continued for a further 12 hr, the precipitated antigen–antibody complex was centrifuged and the radioactivity in the precipitate and supernatant was counted using a Packard γ-spectrometer. The binding experiment results presented are averages of three separate determinations.

Bioassay

The ability of FSH antiserum to achieve specific neutralization of the FSH activity of rat and hamster pituitary was checked in a total gonadotrophin assay. The validity of this assay to ascertain the efficacy of gonadotrophin antiserum to neutralize a gonadotrophin specifically has been described in an earlier communication (Jagannadha Rao & Moudgal, 1970).

RESULTS

Detection of LH antibodies in FSH antiserum by the OAAD test

The results presented in Table 1 show that FSH antiserum freed of antibodies to serum and tissue proteins (as ascertained by the Ouchterlony test) had sufficient antibodies to LH to be able to neutralize LH activity in the

Table 1. Ability of unabsorbed FSH antiserum to neutralize LH activity as tested in the OAAD assay

*Group**	*Treatment LH(μg)*	*Antiserum*	*OAA/100 mg ovary*	*% depletion*	P *OAA*
I	—	NRS	86·7 ± 3·0	—	—
II	1	—	42·6 ± 1·6	50·7	<0·001 (I:II)
III	4	—	20·2 ± 1·5	76·6	<0·001 (I:III)
IV	4	Antiserum (UA)	66·7 ± 3·5	22·8	<0·001 (III:IV)
V	4	Antiserum (A)	22·1 ± 1·3	74·42	<0·05 (III:V)

Female rats (25 days old) were primed with 100 i.u. PMSG and 40 i.u. HCG. The hormone and antiserum were given separately by the intraperitoneal route. The antiserum (0·5 ml) was given immediately after the hormone injection. Autopsy was performed 4 hr after the hormone injection, and the ovaries were processed for ascorbic acid.

NRS = normal rabbit serum (controls); A = absorbed antiserum; UA = unabsorbed antiserum.

* Five animals/group.

$$\% \text{ depletion of OAA} = 100 - \frac{\mu\text{g OAA/100 mg of treated group} \times 100}{\mu\text{g of OAA/100 mg of ovary of saline control}}$$

OAAD test (Table 1, Groups III and IV). Absorption of LH antibodies by the method described (see 'Materials and Methods'), however, removed the contaminating antibodies completely (Table 1, Groups III and V).

Binding studies with ^{125}I-labelled hormones

The ^{125}I-labelled hormone-binding studies, the results of which are shown in Table 2, give a much clearer picture of the efficacy of the absorption procedure used here. Thus, while the FSH antiserum absorbed free of serum

Table 2. Characterization of antisera-labelled hormone binding studies

Group	*Antiserum (a/s)**	*^{125}I-labelled hormone*	*% specific binding*
I	FSH a/s unabsorbed	FSH	58·2
II	FSH a/s unabsorbed	LH	39·4
III	FSH a/s absorbed	FSH	54·7
IV	FSH a/s absorbed	LH	1·4
V	FSH a/s	FSH	29·0
VI	FSH a/s+50 ng LH	FSH	28·0
VII	FSH a/s+1 ng FSH	FSH	11·0
VIII	LH a/s	LH	43·0
IX	LH a/s+absorbed FSH a/s (10 μl)	LH	42·0
X	LH a/s	FSH	89·0
XI	LH a/s	FSH	1·8

See text for details of incubation.

* In Groups I, II, III, IV, X, XI, 10 μl undiluted antiserum was used. Other groups were given 100 μl of 1:1000 diluted antiserum (absorbed).

and tissue proteins is able to bind both [^{125}I]LH and [^{125}I]FSH to a significant extent (Table 2, Groups I and II) following treatment with LH immunosorbent, the binding to LH is reduced almost to zero (Table 2, Group IV). The binding capacity of the antiserum to [^{125}I]FSH, however, remains unchanged (Table 2, Group III). The specificity of the antiserum to bind [^{125}I]FSH was demonstrated by determining the extent of binding in the presence of cold FSH and LH, the latter being present in large excess (Table 2, compare Groups VI and VII with Group V).

The possibility that the use of LH immunosorbent might have resulted in leaching of LH from immunosorbent into the FSH antiserum had also to be checked since it was intended that this antiserum should be used specifically to block FSH without in any way contributing to the LH pool. This was done by determining the binding ability of [^{125}I]LH to LH antiserum in the presence and absence of immunosorbent-treated FSH antiserum. The presence of leached LH in FSH antiserum would have reduced the binding of LH antiserum (Table 2, compare Group VIII with Group IX) to LH.

The specificity of the LH antiserum to bind only LH was also checked using a similar system and the results show clearly the absence of FSH antibodies (Table 2, Groups X and XI).

Ability of FSH antiserum to neutralize rat and hamster FSH

Making use of the fact that total gonadotrophin assay is dependent upon the combined activity of FSH and LH, the ability of FSH antiserum freed of

Table 3. The ability of absorbed FSH antiserum to neutralize the FSH activity of rat and hamster pituitary extract as checked in the total gonadotrophin assay using 21-day-old female mice

Group	*No. of animals*	*Treatment*	*Uterine weight (mg ± S.D.)*
I	5	NRS* 0·5 ml	14·0 ± 1·9
II	5	One adult rat pituitary extract +NRS 0·5 ml	59·0 ± 8·4
III	5	One adult rat pituitary extract +0·5 ml absorbed FSH a/s	14·3 ± 4·4
IV	3	NRS* 0·5 ml	6·5 ± 1·0
V	3	One adult male hamster pituitary extract+0·5 ml NRS	49·1 ± 2·8
VI	3	One adult male hamster pituitary extract+0·2 ml absorbed FSH a/s	16·1 ± 9·1
VII	3	One adult male hamster pituitary extract+0·5 ml absorbed FSH a/s	7·5 ± 0·5

Pituitary extract and antiserum were injected subcutaneously at different sites. Autopsies were performed 24 hr after the last injection.

* Control animals received normal rabbit serum (NRS). a/s = antiserum.

antibodies to serum, tissue proteins and LH to neutralize FSH activity of rat and hamster pituitary extract was demonstrated and the results are presented in Table 3. It can be seen that the quantity of FSH antiserum used was sufficient to neutralize the FSH activity present in the pituitary extract of either rat or hamster.

Ovulation experiments

The ovulation experiments were carried out using three experimental

Table 4. The effect of administration of FSH or LH antiserum on the induction of ovulation in immature rats using either rat pituitary extract or ovine gonadotrophins as ovulatory stimuli

Group	*Treatment*	N_0/N	*Average no. of ova ± S.D.*	P
I	20 i.u PMSG	4/4	0	
II	One rat pituitary extract	4/4	20 ± 6·3	
III	One rat pituitary extract+1·0 ml unabsorbed FSH a/s	1/7	2	
IV	One rat pituitary extract+1·0 ml absorbed FSH a/s	4/4	19 ± 2·5	II:IV > 0·5
V	1·0 ml absorbed FSH a/s	0/3	0	
VI	One rat pituitary extract+0·2 ml LH a/s	0/6	0	
VII	200 μg NIH FSH+0·2 ml LH a/s	0/4	0	
VIII	20 μg LH+0·5 ml absorbed FSH a/s	4/4	22 ± 4·8	II:VIII > 0·1 IV:VIII 0·1 to 0·5

Each rat received 20 i.u. PMSG, followed by ovulating hormone 56 hr later. Autopsy was performed 18 hr after the injection of ovulatory hormone.

N_0 = Number of animals induced to ovulate. N = Number of animals in the group. a/s = antiserum.

Table 5. The effect of FSH and LH antiserum on the induction of ovulation in immature hamsters using hamster pituitary extract or ovine gonadotrophins as ovulatory stimuli

Group	*Treatment*	N_0/N	*Average no. of ova* ± *S.D.*	P
I	20 i.u. PMSG	4/4	0	
II	2 hamster pituitary extract equivalent	4/4	22 ± 5·2	
III	2 hamster pituitary extract equivalent +1 ml unabsorbed FSH a/s	3/3	13 ± 2·7	II:III 0·05 to 0·1 III:IV 0·05 to 0·1
IV	2 hamster pituitary extract equivalent +1·0 ml absorbed FSH a/s	3/3	24 ± 6·8	II:IV > 0·5
V	2 hamster pituitary extract equivalent +0·2 ml LH a/s	0/3	0	
VI	200 μg NIH FSH+0·2 ml LH a/s	0/4	0	
VII	25 μg LH+0·5 ml absorbed FSH a/s	4/4	23 ± 4·8	II:VII > 0·5

Each hamster received 20 i.u. PMSG followed by ovulating hormone 56 hr later. Autopsy was performed 18 hr after injection of ovulatory hormone.
N_0 = Number of animals induced to ovulate. N = Number of animals in the group. a/s = antiserum.

models—the first and second using immature animals, the ovulatory stimulus being of homologous or heterologous origin. In the third model, adult cycling animals were used, the endogenous ovulatory stimulus being neutralized with either FSH or LH antiserum.

It is clear from the data presented in Tables 4 and 5 that the trophic stimulus, whether an homologous pituitary extract or a purified heterologous gonadotrophin, is able to elicit a good ovulatory response in the animals primed with PMSG. While simultaneous treatment with FSH antiserum did not appear to influence ovulation, treatment with LH antiserum in all cases showed complete blockade of ovulation (Table 4, Groups VI and VII and Table 5, Groups V and VI). Attention should be drawn here to the ability of unabsorbed FSH antiserum to block ovulation, an effect which was abolished by 'cleansing' (Tables 4 and 5, Groups III and IV). The fact that FSH antiserum alone is unable to

Table 6. The effect of administration of antiserum to FSH or LH on ovulation at pro-oestrus in adult rats and hamsters

Group	*Treatment*	N_0/N	*Average no. of ova* ± *S.D.*	P
Cycling rats				
I	NRS*	5/5	13·2 ± 2·9	
II	0·2 ml LH a/s	0/6	0	
III	0·5 ml absorbed FSH a/s	6/6	11·0 ± 3·0	I:III > 0·1
Cycling hamsters				
IV	NRS*	5/5	7·4 ± 2·1	
V	0·2 ml LH a/s	0/5	0	
VI	0·5 ml absorbed FSH a/s	5/5	8·2 ± 1·7	IV:VI > 0·5

N_0 = number of animals induced to ovulate. N = number of animals in the group. a/s = antiserum.

* Control animals received 0·5 ml of normal rabbit serum (NRS).

induce ovulation shows that it is free of LH contamination (Table 4, Group V). The system using heterologous preparations is particularly interesting since here an attempt was made to use immunologically 'cleansed' gonadotrophin preparations as ovulatory stimuli. The results clearly suggest that FSH is unable to induce ovulation in the absence of LH (Table 4, Group VII and Table 5, Group VI).

Essentially similar results were obtained using FSH and LH antisera in cycling adult rats and hamsters, LH antiserum being able to block ovulation while FSH antiserum was ineffective in inhibiting the ovulatory process (Table 6).

DISCUSSION

The results of the present investigation have shown that LH is the physiological trigger for ovulation. The ability of FSH to induce ovulation has been checked in various model systems and it can be concluded that, in the absence of LH, FSH alone cannot induce ovulation. In the immature model systems using heterologous gonadotrophins, administration of FSH with LH antiserum at a time appropriate for injection of the ovulatory stimulus does not bring about ovulation. This experiment raises the question of whether LH antiserum, by neutralizing residual PMSG activity, is affecting the ovulatory response of the follicle to concomitantly administered FSH. The results of Sasamoto & Kennan (1972), however, show that neutralization of PMSG at the time of, or 3 hr before, injection of ovulatory hormone does not significantly affect the subsequent response of the follicle to ovulation stimuli.

It is evident from the results presented that although characterized FSH antiserum is able to bind and neutralize FSH specifically, it is unable to influence LH activity. Recent experiments on compensatory hypertrophy of the rat ovary and follicular development in hamsters, in which characterized FSH antiserum has been used, have shown that this antibody is able effectively to neutralize endogenous FSH (A. Jagannadha Rao, C. S. Sheela, S. Prahalad and N. R. Moudgal, unpublished results).

The methods used earlier to absorb FSH antiserum free of LH antibody involved the addition of exogenous LH till all the LH antibodies were removed. In such a method, the possibility of adding excess LH to the antiserum always exists. As seen in some of our recent experiments, the antibody to LH in the FSH antiserum was not always of the precipitating type detectable by the Ouchterlony test. By contrast, the present method permits the removal of both the precipitating and soluble type of antibodies to LH in one step. Further, the FSH antiserum by this method does not get contaminated with excess free LH and its antibody titre to FSH remains unchanged after absorption.

The basic difference between the present study and earlier investigations has been the attempt to achieve an exclusive test of the ovulation-inducing ability of FSH, i.e. in the total absence of endogenous and exogenous LH. It is probable that, under the conditions used by earlier investigators, FSH in some way exerted a permissive or additive influence on the minimal amounts of LH available to effect ovulation. The fact that administration of natural or synthetic LH-RF results in the concomitant release of both LH and FSH from the

pituitary (Guillemin, 1971) may invalidate the idea that the appearance of FSH with LH in surge form would mean a rôle for the former in the ovulation process. The appearance of FSH in surge form just before ovulation recalls the suggestion of Schwartz (1969) that it may initiate fresh folliculogenesis for a subsequent cycle.

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