Development in the cyclic hamster of refractoriness to the superovulatory action of anti-LH serum

G. S. Greenwald and P. F. Terranova

Ralph L. Smith Research Center, Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A.

Summary. Hamsters injected s.c. on the day of ovulation (Day 1) with 100 μ l equine anti-bovine LH serum ovulated 28 eggs at the end of a 5-day cycle. When a second injection of anti-LH serum was administered 4–93 days later, the animals did not superovulate and had normal 4-day cycles. Injection of 100 μ l normal rabbit serum (NRS) on Day 1 followed 14 days later by anti-LH serum resulted in the ovulation of 32 ova whereas a priming injection of 100 μ l normal horse serum (NHS) followed by anti-LH serum resulted in the ovulation of only 18 ova. When hamsters were injected on Day 1 with anti-LH serum, NHS or NRS and then with anti-LH serum in the 4th cycle, high titres of free antibodies to LH were present on Days 2–4 only in the animals treated with NRS; these hamsters ovulated a mean of 35 ova.

These experiments suggest that the hamster rapidly forms antibodies to equine immunoglobulins, thus preventing a second injection of anti-LH serum from inducing superovulation.

Introduction

A single s.c. injection of equine anti-bovine LH serum (1) induces superovulation of 30–35 eggs in pregnant or cyclic hamsters (Terranova & Greenwald, 1979a; Greenwald & Terranova, 1981); (2) increases the ovulation rate from 3 to 6 in the cyclic guinea-pig (Terranova & Greenwald, 1981a); but (3) is unable to increase the number of ovulations (above the normal 10–12) in the pregnant rat (Terranova & Greenwald, 1981b). Although the cyclic hamster superovulates after anti-LH treatment, the concentrations of serum oestradiol are not abnormally elevated, as is the case after administration of PMSG (Baranczuk & Greenwald, 1973).

Our findings with regard to anti-LH treatment in the hamster may be applicable to the induction of superovulation in cows and sheep. However, to be practical for use in animal husbandry it should be possible to increase the ovulation rate in the same animal in several different cycles. Accordingly, the present study was designed to establish whether cyclic hamsters treated with anti-LH serum would superovulate in response to a second injection of the antiserum.

Materials and Methods

Female hamsters (*Mesocricetus auratus*), weighing 80–120 g, were maintained in rooms with a lighting schedule of 14 h light–10 h dark (lights on at 05:00 h) and used after 3 successive 4-day oestrous cycles, as monitored by the conspicuous post-ovulatory vaginal discharge present on Day 1 of the cycle. Day 1 corresponds to the morning of ovulation (= oestrus) and Day 4 to pro-oestrus.

The equine anti-bovine LH serum was produced in a mare and was generously provided by Dr William Hansel, Cornell University. We have previously characterized the antiserum (Terranova & Greenwald, 1979a) using the methods described by Morishige & Rothchild (1974). The binding with ^{125}I -labelled rat FSH was approximately 3% of that of rat LH and binding of ^{125}I -labelled rat prolactin did not differ from non-specific binding values (Terranova & Greenwald, 1979a).

Experiment I. Hamsters were injected s.c. at 09:00 h on Day 1 of the cycle (Cycle 1) with 100 μl anti-LH serum: the first group of animals were killed at 09:00 h on Day 6, the oviducts flushed and newly ovulated ova counted. For subsequent groups, the initial injection of anti-LH serum was followed by a second injection of 100 μl anti-LH serum at 09:00 h on Day 1 of the second to 24th cycle (i.e. 5–93 days after the first injection). The animals were then killed on Day 1 of the next cycle and tubal ova were counted.

Experiment II. Hamsters were injected s.c. on Day 1 of the first cycle with 100 μl anti-LH serum or, as controls, with 20 i.u. PMSG, 100 μl normal horse serum (NHS) or 100 μl normal rabbit serum (NRS). This was followed by a second injection on Day 1 of the 4th cycle of 100 μl anti-LH serum, NHS or 20 i.u. PMSG. The animals were killed on Day 1 of the 5th cycle and the oviducts flushed and ova counted. The ovulation rate of 7 untreated hamsters was also checked.

Experiment III. To determine the titre of antibody to LH, groups of hamsters were injected s.c. with 100 μl anti-LH serum on Day 1 and decapitated on Days 4, 8, 12 and 16 ($N = 4$ for each day). Trunk blood was saved for determination of serum unbound (free) anti-LH by the procedure of Morishige, Billiar & Rothchild (1975) as utilized by Terranova & Greenwald (1979b). The radiolabelled gonadotrophin was ^{125}I -labelled ovine LH.

Experiment IV. Four groups of hamsters ($N = 8$ per group) were injected s.c. on Day 1 of Cycle 1 with 100 μl anti-LH serum, NRS or NHS. This was followed by a second s.c. injection of 100 μl NHS or anti-LH serum at 09:00 h on Day 1 of the 4th cycle. Immediately before the second injection, the unanaesthetized hamsters were bled by cardiac puncture to recover 0.1–0.2 ml blood for determination of the titres of free antibodies to LH. Subsequently, the animals were bled at 09:00 h on Days 2, 3, 4 and 5 and decapitated on Day 6, and the serum levels of free antibodies to LH were measured. In addition the oviducts were flushed and ova counted.

For all experiments, analysis of variance was followed by Duncan's multiple range tests with differences judged significant if $P < 0.05$.

Results

Experiment I. Injection of anti-LH serum resulted in the ovulation of 28 ova with the cycle lengthened to 5 days in all animals (Table 1). A second injection of anti-LH serum, even if 2–3 months later, never resulted in superovulation and in all instances cycle length was constant at the normal 4-day duration.

Experiment II. Treatment with 100 μl NRS, followed by anti-LH serum at the beginning of Cycle 4, resulted in the ovulation of an average of 32 ova (Table 2, Group 1). On the other hand, a priming injection of NHS (Group 2) was as effective as anti-LH serum (Group 4) in negating the superovulatory ability of a second injection of anti-LH serum; the animals ovulated the normal number of 10–13 ova and the cycle lasted 4 days. Injection of anti-LH serum at Cycle 1 did not interfere with the ability of 30 i.u. PMSG to induce superovulation at the end of Cycle 4 (Group 5). Similarly, PMSG administered at Cycle 1 did not prevent a second injection of PMSG from tripling the ovulation rate compared to control animals (Groups 6 & 3).

Experiment III. When hamsters were injected with 100 μl anti-LH serum at 09:00 h on Day 1, the serum titre of free antibodies to LH had dropped to 273 ± 33 nl/ml by Day 4, to 67 ± 22 nl/ml by Day 8 and to 16 ± 5 nl/ml by Day 12. The concentration of antibodies to LH was virtually undetectable by Day 16 (5 ± 2 nl/ml).

Experiment IV. Priming injections at Cycle 1 of anti-LH serum or NHS (Groups 2 & 4, Table 3) prevented the superovulatory action of anti-LH serum in Cycle 4. Normal rabbit serum injected at

Table 1. Effects of a second injection of anti-LH serum on ovulation by the cyclic hamster

Anti-LH serum (100 µl) on Day 1 of:	Interval (days) between 1st and 2nd injection of anti-LH serum	No. of ovulations
Cycle 1	—	27.7 ± 2.8 (6)
Cycles 1 & 2	5	11.2 ± 1.6 (6)*
Cycles 1 & 3	8–9	14.9 ± 1.4 (8)*
Cycles 1 & 4	14–15	12.1 ± 1.2 (3)*
Cycles 1 & 5	29–30	11.7 ± 0.7 (7)*
Cycles 1 & 16	60–61	10.2 ± 1.4 (6)*
Cycles 1 & 24	91–93	5.8 ± 1.4 (5)*

Values are mean ± s.e.m. for the no. of hamsters given in parentheses.

* $P < 0.01$ compared with value for Cycle 1.

Table 2. Number of ovulations in hamsters after various treatments

Group	Treatment† at Day 1 of:		No. of ovulations
	Cycle 1	Cycle 4	
0	—	—	13.1 ± 0.6 (7)
1	Normal rabbit serum	Anti-LH serum	32.2 ± 2.9 (6)
2	Normal horse serum	Anti-LH serum	17.6 ± 1.7 (5)*
3	Anti-LH serum	Normal horse serum	13.3 ± 0.8 (6)*
4	Anti-LH serum	Anti-LH serum	13.2 ± 1.5 (6)*
5	Anti-LH serum	PMSG	42.4 ± 3.7 (6)*
6	PMSG	PMSG	40.8 ± 3.6 (6)**

† Each s.c. injection = 100 µl.

Values are mean ± s.e.m. for the no. of hamsters given in parentheses. Compared with value for Group 1; * $P < 0.01$, ** $P < 0.05$.

Table 3. Number of ovulations in hamsters at the end of the 4th cycle after various priming injections at Cycle 1

Group	Treatment† at Day 1 of:		No. of ovulations on Day 1 of Cycle 5
	Cycle 1	Cycle 4	
1	Anti-serum	Normal horse serum	13.3 ± 0.8 (6)*
2	Anti-LH serum	Anti-LH serum	13.3 ± 1.3 (6)*
3	Normal rabbit serum	Anti-LH serum	34.6 ± 2.4 (7)
4	Normal horse serum	Anti-LH serum	17.8 ± 0.8 (8)*

† Each s.c. injection = 100 µl.

Values are mean ± s.e.m. for the no. of hamsters given in parentheses.

* $P < 0.01$ compared with value for Group 3.

Cycle 1 did not interfere with the activity of anti-LH serum and the cycle lasted 5 days (Group 3). After injection of NHS at Cycle 4, the hamsters ovulated the normal number of ova at the end of a 4-day cycle (Group 1).

The temporal changes in unbound LH antibodies in the above animals are shown in Text-fig. 1. After an initial injection of NRS, titres of LH antibodies were high on Day 2 with a gradual decrease over the next 4 days. In contrast, in the groups primed with NHS or anti-LH serum, the antibody titres were 32–43% of those primed with NRS and by Day 3 and thereafter the titres of the former two groups challenged with anti-LH serum did not differ significantly. Finally, the animals injected with anti-LH serum during Cycle 1, followed by NHS, had very low levels of LH antibodies (18 to 8 nl/ml serum decreasing from Days 1 to 6).

Text-fig. 1. Serum levels of free antibodies to LH on Days 2–6 after the injection of 100 μ l anti-LH serum on Day 1 of Cycle 4. The priming injection on Day 1 of Cycle 1 consisted of anti-LH serum, normal horse serum (NHS) or normal rabbit serum (NRS). Values are mean $\pm$ s.e.m. for 8 hamsters/group.

Discussion

These experiments show that after a priming injection of anti-LH serum, subsequent treatment with the antiserum never resulted in superovulation. Moreover, normal horse serum, but not normal rabbit serum, was equally effective in preventing anti-LH serum from inducing superovulation. The common denominator was that both normal horse serum and anti-LH serum contain equine immunoglobulins. However, exposure first to normal horse serum did not interfere with the ability of another equine preparation, PMSG, from eliciting a 3-fold increase in the ovulation rate.

After an initial injection of anti-LH serum, the titres of unbound LH antibodies in serum dropped rapidly. Hence, the refractoriness to a second injection of anti-LH serum by Cycles 3 or 4 can not be attributed to continuing high titres of LH antibodies having long-term consequences on follicular development. In fact, after a single injection of anti-LH serum, subsequent cycles are restored from the second cycle to the normal 4-day duration and the animals ovulate 10–13 ova (Greenwald & Terranova, 1981).

How then can we explain the present results? The most likely hypothesis is that after the primary exposure to equine anti-bovine LH, the hamster rapidly develops antibodies to equine immunoglobulins; a secondary exposure to anti-LH serum results in an immediate build up of hamster IgG and IgM which destroy the foreign antigens. Hence, the titres of unbound LH antibodies never reach the levels necessary to neutralize completely the circulating LH which we believe is necessary for superovulation to occur (Terranova & Greenwald, 1981c). This interpretation is consistent with the data shown in Text-fig. 1.

Female hamsters injected with sheep red blood cells show a much greater primary immune response than do males in the form of IgG and IgM plaque-forming cells from the spleen (Blazkovec, Orsini & Maginn, 1973). Similarly, when given a priming injection of sheep red blood cells followed 31 days later by a challenging dose of the same antigen, the secondary immune response of female hamsters is much greater than for the male (Orsini & Blazkovec, 1974). The next obvious study with anti-LH serum is to determine whether there is a secondary immune response in hamsters receiving a challenging injection of the antiserum. The problems encountered in increasing the ovulation rate in hamsters beyond the first cycle of treatment with anti-LH serum may be overcome in hamsters, or farm animals, by passive immunization with antiserum prepared in the homologous species.

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