Ovulatory response of hypophysectomized hamsters to deferred injection of LH

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Summary. Follicles failed to respond to the ovulating hormone stimulus (5 or 25 µg LH) when this was given 8 h after hypophysectomy. Shorter delays resulted in full or partial (<6 ova) ovulation.

Introduction

Follicular atresia is an intriguing but poorly understood phenomenon mainly because it is usually recognized retrospectively and seemingly occurs as a random, sporadic event. The development of animal models in which degeneration, especially of antral stages, can be produced in a synchronized population of follicles, offers an attractive approach to analysing various aspects of atresia. I have previously proposed that hamsters hypophysectomized and injected immediately with PMSG, followed 2–3 days later by intraperitoneal injection of an antiserum to PMSG could provide such a model (Greenwald, 1963). Administration of 30 i.u. PMSG to hypophysectomized hamsters results in the maturation of 40–50 antral follicles per animal (Greenwald, 1973a) and very high (~700 pg/ml) blood levels of oestradiol (Greenwald, 1973b). The oestradiol levels fall to 40 pg/ml by 7 h after treatment with the antiserum to PMSG (Greenwald, 1973b).

This model provides a convenient method of inducing atresia in large numbers of antral follicles, but suffers from the possible disadvantage that excessively high steroid levels are present. In the present approach atresia was induced by hypophysectomizing hamsters at pro-oestrus, when ripe antral follicles are present, and investigating for how long mature antral follicles can be deprived of gonadotrophins and still ovulate in response to exogenous LH.

Materials and Methods

The hamsters (*Mesocricetus auratus*) were maintained under standard lighting conditions of 14 h light:10 h dark (lights on 05:00–19:00 h). At 13:00 h (± 10 min) on the day of pro-oestrus (2 h before the LH surge) the animals were hypophysectomized and, as shown in Table 1, were injected intraperitoneally at various times with 5 (Groups 3–10) or 25 (Group 11) µg ovine LH (NIH-LH-S13) in a volume of 0.1 ml physiological saline (0.154 M-NaCl). The control, pro-oestrous hamsters were intact and untreated (Group 1) or hypophysectomized at 13:00 h and injected immediately with 0.1 ml saline (Group 2).

The animals were killed 12–24 h after hypophysectomy with a minimal time of 12 h elapsing after the LH injection. The oviducts were flushed with physiological saline to recover ovulated eggs. The sella turcica was examined under a dissecting microscope and any animals with visible fragments of pituitary tissue were discarded. Statistical significance was examined by Student's *t* test.

Results

As shown in Table 1, none of the hypophysectomized and saline-injected hamsters in Group 2 had ovulated. Ovulation occurred in Groups 3–9 but the numbers ovulating and the mean numbers of eggs ovulated decreased with time. Even when the amount of LH was increased, ovulation did not occur in response to injection at 21:00 h.

Table 1. Ovulatory response of hamsters to ovine LH after hypophysectomy (hypox) at 13:00 h on the day of pro-oestrus

Group	Treatment	No. of hamsters with:			Mean ($\pm$ s.e.m.) no. of ovulations†
		Full ovulation (>8 ova)	Partial ovulation (<6 ova)	No ovulation	
1	Intact, unoperated	8	0	0	13.4 $\pm$ 0.7 (8)
2	Hypox + saline	0	0	7	0
	Hypox +				
3	5 μ g LH, 13:00 h	8	0	0	12.5 $\pm$ 0.6 (8)
4	5 μ g LH, 14:00 h	8	0	0	11.1 $\pm$ 0.6 (8)*
5	5 μ g LH, 15:00 h	5	2	1	9.3 $\pm$ 1.6 (7)*
6	5 μ g LH, 16:00 h	4	3	0	7.3 $\pm$ 2.0 (7)**
7	5 μ g LH, 17:00 h	1	2	5	4.7 $\pm$ 2.0 (3)**
8	5 μ g LH, 19:00 h	2	1	5	8.0 $\pm$ 2.5 (3)
9	5 μ g LH, 20:00 h	2	3	3	5.6 $\pm$ 2.0 (5)***
10	5 μ g LH, 21:00 h	0	0	8	0
11	25 μ g LH, 21:00 h	0	0	4	0

Values significantly different from those in Group 1: * $P < 0.05$, ** $P < 0.025$, *** $P < 0.005$.

† Of animals with partial and full ovulation.

Discussion

The present results show that hamster follicles fail to respond to the ovulating hormone stimulus when this is given 8 h after hypophysectomy (Table 1). In the pregnant hamster, all antral follicles show histological signs of atresia by 6 h after a single injection of anti-LH serum (P. Terranova & G. S. Greenwald, unpublished). Similarly, in pro-oestrous hamsters hypophysectomized between 08:00 and 11:00 h, the first signs of atresia in antral follicles appears in the granulosa cells between 16:00 and 20:00 h (D. D. Chiras & G. S. Greenwald, unpublished). Antral follicles of hamsters are therefore unable to ovulate after deprivation of LH for 6–8 h.

The only other report comparable to the present study involved the rat and showed that by 8 and 10 h after hypophysectomy 87 and 44% respectively of the animals had ovulated after intravenous injection of hCG, while by 12 h only 1 of 8 similarly treated rats ovulated (Talbert, Meyer & McShan, 1951). The number of ovulations was not determined for the hCG-treated rats, but it may be concluded that follicular atresia occurs sooner after hypophysectomy in hamsters than in rats.

One of the possible objections to experiments involving hypophysectomy is that the entire endocrine system is disturbed and that the failure to ovulate could be compounded by insufficiency of FSH, prolactin or even ovarian steroids. Therefore, detailed studies, measuring the acute changes in peripheral and ovarian hormone levels, are necessary before the validity of this model can be established in the analysis of follicular atresia.

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