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In *vitro* responsiveness of hamster corpora lutea undergoing luteolysis to luteinizing hormone

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Abstract. Corpora lutea removed from pregnant hamster deprived of endogenous luteinizing hormone for varying periods were compared for their responsiveness to externally added luteinizing hormone. The corpora lutea removed on the 8th day of pregnancy exhibited a dose-dependent increase in progesterone production in response to added luteinizing hormone upto a concentration of 2·5 μg/ml. The total progesterone synthesised by the corpora lutea decreased with increase in the duration of *in vivo* luteinizing hormone deprivation. However, the hormone deprivation had to be for a minimum period of 24 h before a marked reduction in the *in vitro* responsiveness could be seen. Neutralisation of endogenous luteinizing hormone increased the luteal cholesterol ester concentration, while *in vitro* incubation of such tissue with luteinizing hormone resulted in a marked reduction in cholesterol ester levels. Corpora lutea removed from hamsters on day 8, 15 and 16 of pregnancy when compared for their responsiveness *in vitro* to added luteinizing hormone showed that the luteal tissue of day 8 produced more progesterone relative to those of day 15/16. In contrast, depletion of free and esterified cholesterol increased with the increase in age of corpora lutea (from 15% on day 8 to 67% on day 16).

Keywords. Luteolysis; luteinizing hormone; progesterone; hamster

Introduction

Luteinizing hormone (lutropin, LH) stimulates steroidogenesis *in vivo* and *in vitro* in functional corpora lutea of a variety of species (Armstrong, 1968; Savard, 1973; Dorfman, 1972; Sairam and Li, 1973). This stimulation of steroidogenesis in many cases has been correlated with reduction in cholesterol concentration (Behrman and Armstrong, 1969; Marsh, 1976). Conversely, the corpora lutea undergoing luteolysis show little or no response to LH. Thus, in bovine luteal slices, the response to LH was lost between days 16 and 18 of the 22–day estrous cycle (Armstrong and Black, 1966). Similarly, *in vitro* addition of LH to corpora

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lutea from heifers treated *in vivo* with luteolytic doses of estradiol resulted in a lowered progesterone synthesis (Hansel *et al.,* 1973). In addition, when LH was added to corpora lutea undergoing lyteolysis, there was a decrease in adenylate cyclase activity resulting in lowered levels of cyclic AMP (cAMP) (Anderson *et al.,* 1974; Muralidhar and Moudgal, 1976).

We have recently reported that deprivation of endogenous LH by administration of anti-LH serum (LH a/s) on day 7 or 8 of pregnancy in the hamster caused luteolysis as judged by reduction in serum progesterone levels and accumulation of cholesterol esters (Mukku and Moudgal, 1975). The effect of *in vitro* addition of LH on progesterone synthesis and cholesterol levels in corpora lutea obtained from control and LH-deprived hamsters during different stages of pregnancy is reported in this communication.

Materials and methods

Luteinizing hormone antiserum used in this study was raised against ovine LH in monkeys. The methods used for characterisation, determination of cross-reactivity and potency of the antiserum were essentially the same as those described in detail earlier (Madhwa Raj and Moudgal, 1970).

Hamsters were injected with 100 μl of LH a/s (intracardiac) on day 7 or 8 of pregnancy, 3 and 24 h prior to autopsy time on day 8. The amount of a/s given was sufficient to neutralise circulating LH for more than 24 h. Corpora lutea were separated, quickly weighed and incubated in Eagle's minimal medium which was buffered to pH 7·4 with HEPES salt containing glutamine and bicarbonate. The animals were killed under light ether anesthesia, ovaries quickly dissected out, rinsed in ice-cold saline and the corpora lutea were immediately separated from the non-luteal tissue. This process was completed within 3 min for each animal. Corpora lutea from 3 or 4 animals were pooled, randomly distributed and after light blotting, each group was weighed to 0·1 mg accuracy. They were then transferred to incubation flasks containing 1 ml of ice-cold medium, and LH (NIH-LH-S-19, 5-10 μg was added. Each flask was then flushed with oxygen for 10 s, tightly stoppered and incubated at 37° C in a Dubnoff metabolic shaker. Whenever needed (10 μl*)* aliquots of the medium were removed at different time intervals for progesterone assay. At the end of the incubation period, the medium diluted appropriately with the assay buffer was used directly without extraction for estimation of progesterone. Progesterone in the luteal tissue was measured by radioimmunoassay after homogenisation of the tissue and extraction of the steroid by diethyl ether as described earlier (Mukku and Moudgal, 1975, 1976).

For assaying cholesterol, the tissue along with the medium in each flask was homogenised in a Potter-Elvehjem homogeniser, extracted with chloroform methanol (2:1) and separated by thin layer chromatography (TLC) as described by Major *et al.* (1967) and Pokel *et al.* (1972). Free and esterified cholesterol were estimated spectrophotometrically using H_2SO_4 – $FeCl_3$ reagent as described by Glick *et al.* (1964).

Results

Kinetics of progesterone production in functionally active corpora lutea of pregnant hamster

In vitro incubation of corpora lutea of day 8 pregnant hamsters with LH increased the progesterone concentration in the medium and tissue with time and this reached a plateau at 2 h. Incubation in all subsequent experiments was therefore carried out for 2 h only. Progesterone production linearly increased with the concentration of LH with maximum effect at 2·5 μg LH/ml. At concentrations of 10 and 20 μg of LH/ml, the total progesterone production was reduced; while the progesterone level of the medium remained unaltered, a marked decrease in the concentration in the tissue was observed (figure 1).

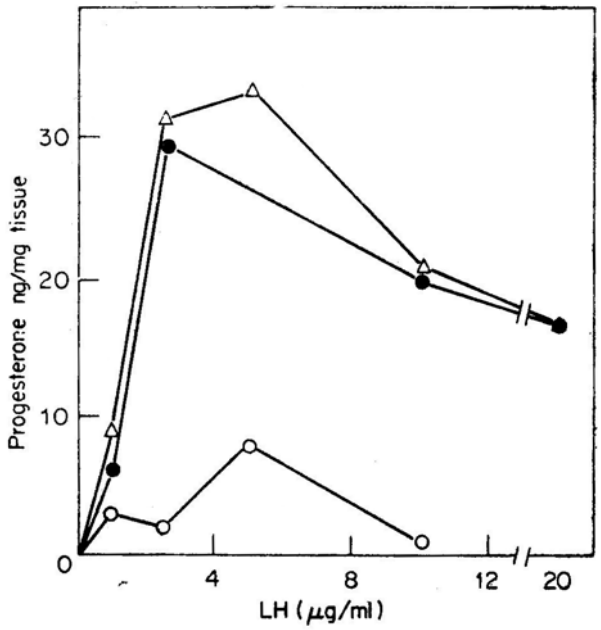

Figure 1. Effect of LH concentration in the medium on the ability of day 8 pregnant hamster corpora lutea to synthesise and secrete progesterone. Corpora lutea from 6 hamsters were pooled and randomly distributed into 6 flasks and incubated in 1 ml of the medium for 2 h with LH at the indicated concentrations. Progesterone produced in *vitro* in the absence of LH has been deducted from all values O—O tissue, ●—● medium and Δ—Δ tissue and medium.

Effect of in vivo LH deprivation on progesterone production by corpora lutea of pregnant hamsters in vitro

The time course of progesterone secretion into the medium by the corpora lutea of day 8 pregant hamsters deprived of endogenous LH support for 0, 3, 6, 12 and 24 h is shown in figure 2. It is evident that the total progesterone secreted into the medium over a 2 h period is markedly decreased only when the duration of LH deprivation *in vivo* is 12 h or more. However, the stimulatable progesterone

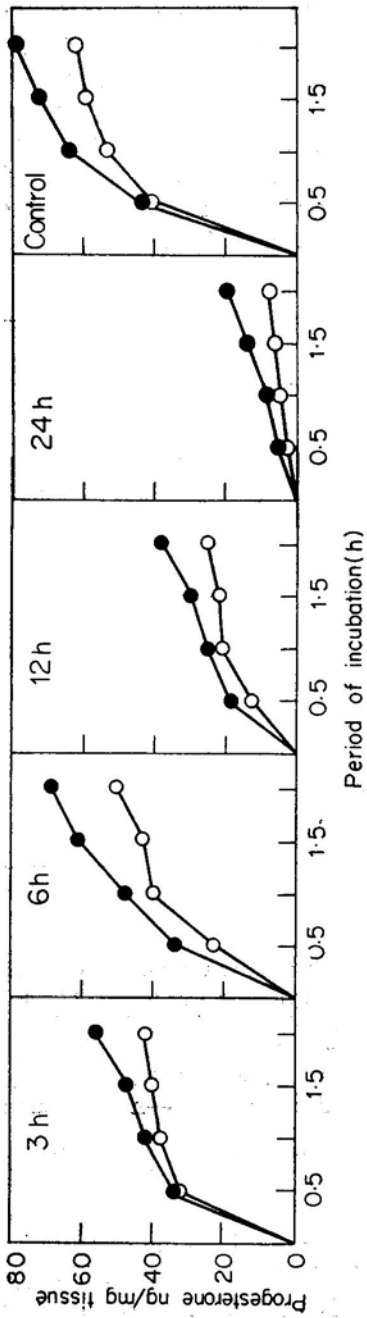

Figure 2. Time course of progesterone secretion into the medium by the corpora lutea of day 8 pregnant hamster after LH-deprivation *in vivo*. The numbers 3, 6, 12 and 24 h refer to hours of LH-deprivation *in vivo* before being incubated *in vitro*. Each point is a mean of two experiments and in each experiment corpora lutea from three animals were distributed into the flasks, one incubated without LH (–○–), and the other with LH 2·5 μg/ml (–●–). Secretion of progesterone into the medium at '**O**' h was considered to be zero.

synthesis *in vitro* appears to be markedly altered only after endogenous LH is neutralised for 24 h.

Ability of LH to stimulate in vitro hydrolysis of esterjfied cholesterol in corpora lutea removed from LH antiserum-treated hamsters : It is evident from table 1 that the levels of free and esterified cholesterol in corpora lutea of hamsters deprived of *in vivo* LH support for 24h, are greatly reduced following *in vitro* incubation with LH. Deprivation of LH for shorter periods, particularly for 6 h or less had only marginal effect on subsequent sterol depletion seen following incubation with added LH.

Responsiveness in vitro of corpora lutea undergoing natural luteolysis to added LH : Luteolysis in corpora lutea occurs in the hamster on day 15 and 16 of pregnancy. In the present study the responsiveness of these corpora lutea to added LH was compared with that of functionally active corpora lutea obtained on the 8th day of pregnancy.

The amount of progesterone synthesised in response to added LH decreased from 31 ng/2h on day 8 to 10 ng/2h on day 15. The amount synthesised by day 16 corpora lutea (removed 4–5 h after parturition) was negligible (table 2). The difference between the unincubated and incubated controls of day 16 corpora lutea was however, significant (1 6 ng progesterone/mg/2 h vs 9·0 ng/progesterone/mg/2 h). There was a marked decrease in the amount of cholesterol ester when

Table 1. The *in vitro* depletion of cholesterol and its esters on the addition of LH to corpora lutea of 8-day pregnant hamsters.

LH-deprivation (h)		Ester cholesterol μg/mg tissue		Free cholesterol μg/mg tissue	
		Without LH	With LH*	Without LH	With LH*
0	I	0·66	0·56	0·66	0·48
	II	1·10	0·92	1·51	1·20
3	I	0·88	0·73	1·47	1·52
	II	1·05	0·95	1·60	1·49
6	I	1·34	1·02	1·65	1·70
	II	1·30	0·95	1·51	1·43
12	I	1·72	0·84	0·97	0·52
24	I	3·54	1·83	2·72	1·59
	II	3·81	1·65	2·85	1·50

I and II refer to two separate but identical experiments. In all cases incubation was for 2 h and tissue was from 3 animals.

* 2·5 μg NIH-LH-S19.

Table 2. Effect of LH on progesterone synthesis by corpora lutea of pregnan hamsters.

	Incubation conditions	Progesterone (ng/mg/2 h)	LH stimulatable progesterone synthesis (ng/mg/2 h)
Day 8 pregnancy	Unincubated	33	
	−LH	75	
	+LH 2·5 μg/ml	101	26
	Unincubated	38	
	−LH	69	
	+LH 5·0 μg/ml	106	37
Day 15 pregnancy	Unincubated	29	
	−LH	53	
	+LH 2·5 μg/ml	61	8
	Unincubated	36	
	−LH	63	
	+LH 5·0 μg/ml	75	12
Day 16 pregnancy	Unincubated	1·4	
	−LH	8·6	
	+LH 2·5 μg/ml	8·8	0·2
	Unincubated	1·8	
	−LH	9·5	
	+LH 5·0 μg/ml	10·3	0·8

For incubation conditions. see text. Corpora lutea pooled from 3-4 hamsters were used in each experiment.

corpora lutea of day 15 or 16 pregnant hamsters were incubated with LH, while only marginal reduction was observed with 8 day old corpora lutea. Free cholesterol level of only 16 day old corpora lutea was observed to be reduced following incubation with LH (table 3).

Discussion

The principal function of the corpus luteum is to produce progesterone from its precursors in response to LH stimulation and as such the onset of luteolysis should be indicated by the absence of this response. The day 15 and 16 pregnant hamster corpora lutea are on the verge of luteolysis. A comparison of the metabolic capabilities of these corpora lutea with those of day 8 representing functionally active state, shows that (a) the ability to synthesise progesterone in reponse to LH stimulus *in vitro* is drastically reduced with the advancement of luteal age and (b) cholesteryl ester levels are significantly increased in day 15 and 16 corpora

Table 3. Changes in the amount of esterified and free cholesterol levels in corpora lutea of pregnant hamsters on the addition of LH.

	Esterified cholesterol μg/mg tissve		Free cholesterol μg/mg tissue	
	Without LH	With LH*	Without LH	With LH*
Day 8				
I	0·66	0·56	0·66	0·48
II	1·10	0·92	1·51	1·20
Day 15				
I	2·52	1·43	1·85	1·15
II	2·10	1·40	1·51	0·93
Day 16				
I	3·56	0·95	2·72	1·59
II	2·95	1·20	1·50	0·35

I and II refer to two separate but identical experiments, tissue from 3 animals being used in each experiment. Incubation in all cases was for 2h.

* NIH–LH–S19, 2·5 μg/ml.

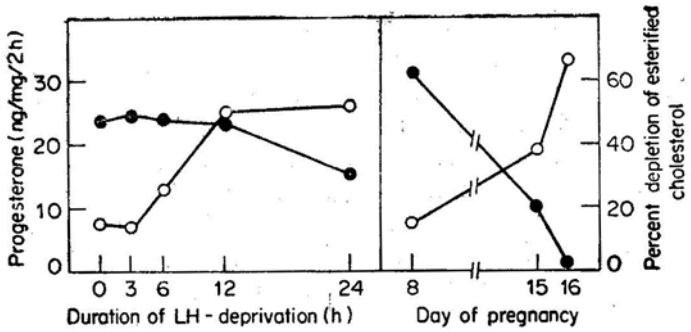

Figure 3. A composite figure showing the progesterone synthesis and percent depletion of esterified cholesterol in response to LH *in vitro* in the corpora lutea of pregnant hamsters. The values are calculated from the data in tables 1 to 3.
–●—●– progesterone synthesised in response to L H .
–O– percent depletion of esterified cholesterol in response to LH.

lutea compared to that in the corpus luteum of day 8 of pregnancy. Normally depletion in free and esterified cholesterol levels is correlated with increase in steroidogenesis. The observation that decrease in free and esterified cholesterol concentration in response to LH occurs in the absence of an increase in progesterone synthesis (tables 1 and 2) in lytic luteal tissue is interesting but difficult to explain.

Following LH-deprivation *in vivo* the luteal tissue essentially behaves like one undergoing natural luteolysis in terms of the biochemical correlates mentioned above. The changes which are similar in both cases appear progressive, the luteal tissue obtained following 24 of *in vivo* LH-deprivation being essentially comparable to that of day 15 or 16 of pregnancy (figure 3). This would perhaps suggest that during natural luteolysis also an apparent localised LH-deprivation confined to the ageing corpora lutea must be occurring. Such selective deprivation could occur due to (a) appearance, at the later stages of luteal phase, of growing follicles in the ovary having a relatively higher avidity and capacity to pick up LH from circulation and/or (b) natural luteolytic agents like prostaglandins, estradiol etc., bringing about their action by reducing luteal LH recreptor activity. Presently evidence is available for only the latter (Behrman and Hichens, 1976 and Grinwich *et al.,* 1976).

It appears from the LH antiserum experiments that once luteolysis has set in (minimal LH deprivation for 12 h) it is difficult to reverse the process. Reversal was attempted by giving to hamsters deprived of endogenous LH support (LH antibody treated for specified periods) either excess LH or a second antibody. This treatment, however, did not restitute luteal functionality, luteal progesterone levels continuing to be reduced (Mukku and Moudgal, unpublished observations). Following LH antiserum injection to pregnant hamsters, luteal cholesterol ester levels relatively raise at a faster rate compared to the fall in serum/luteal progesterone concentration (Mukku and Moudgal, 1975). We have also earlier shown in rats that LH deprivation leads to an inhibition in cholesterol esterase activity and an increment in cholesterol ester synthetase activity, these contributing together perhaps to the overall accumulation of cholesterol esters (Behrman *et al.,* 1971). In the present study, comparing two positive responses to LH stimulation like ester hydrolysis and progesterone synthesis it is observed that they get delinked in a luteal tissue undergoing lysis.

The ability of lytic corpora lutea, whether they be produced by antiserum treatment or ageing process, not only to accumulate cholesterol esters but hydrolyse them when challenged with LH is enigmatic. Considering that corpora lutea, synthesising progesterone maximally (for e.g. that of day 8 of pregnancy), hydrolyse cholesterol esters only to the extent of 15%, it is surprising that lytic corpora lutea not making any progesterone are capable of hydrolysing over 50% of stored, cholesterol esters. The possibility that this is being channelled to produce steroids other than progesterone, though unlikely, is yet to be investigated.

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