J Biosci., Vol. 21, Number 4, June 1996, pp 497-510. © Printed in India.

Assessment of luteal rescue and desensitization of macaque corpus luteum brought about by human chorionic gonadotrophin and deglycosylated human chorionic gonadotrophin treatment

N SELVARAJ, R MEDHAMURTHY, S G RAMACHANDRA, M R SAIRAM* and N R MOUDGAL†

Primate Research Laboratory, Centre for Reproductive Biology and Molecular Endocrinology, Indian Institute of Science, Bangalore 560012, India

*Reproduction Research Laboratory, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

MS received 24 January 1996

Abstract. The objective of the current study was to investigate the mechanism by which the corpus luteum (CL) of the monkey undergoes desensitization to luteinizing hormone following exposure to increasing concentration of human chorionic gonadotrophin (hCG) as it occurs in pregnancy. Female bonnet monkeys were injected (im) increasing doses of hCG or dghCG beginning from day 6 or 12 of the luteal phase for either 10 or 4 or 2 days. The day of oestrogen surge was considered as day '0' of luteal phase. Luteal cells obtained from CL of these animals were incubated with hCG (2 and 200 pg/ml) or dbcAMP (2.5, 25 and 100 μM) for 3h at 37°C and progesterone secreted was estimated. Corpora lutea of normal cycling monkeys on day 10/16/22 of the luteal phase were used as controls. In addition the *in vivo* response to CG and deglycosylated hCG (dghCG) was assessed by determining serum steroid profiles following their administration. hCG (from 15-90 IU) but not dghCG (15-90 IU) treatment *in vivo* significantly ($P < 0{\cdot}05$) elevated serum progesterone and oestradiol levels. Serum progesterone, however, could not be maintained at a elevated level by continuous treatment with hCG (from day 6-15), the progesterone level declining beyond day 13 of luteal phase. Administering low doses of hCG (15-90 IU/day) from day 6-9 or high doses (600 IU/day) on days 8 and 9 of the luteal phase resulted in significant increase (about 10-fold over corresponding control $P < 0{\cdot}005$) in the ability of luteal cells to synthesize progesterone (incubated controls) *in vitro*. The luteal cells of the treated animals responded to dbcAMP ($P < 0{\cdot}05$) but not to hCC added *in vitro*. The *in vitro* response of luteal cells to added hCG was inhibited by 0, 50 and 100% if the animals were injected with low (15-90 IU) or medium (100 IU) between day 6-9 of luteal phase and high (600 IU on day 8 and 9 of luteal phase) doses of dghCG respectively; such treatment had no effect on responsivity of the cells to dbcAMP. The luteal cell responsiveness to dbcAMP *in vitro* was also blocked if hCG was administered for 10 days beginning day 6 of the luteal phase. Though short term hCG treatment during late luteal phase (from days 12-15) had no effect on luteal function, 10 day treatment beginning day 12 of luteal phase resulted in regain of *in vitro* responsiveness to both hCG ($P < 0{\cdot}05$) and dbcAMP ($P < 0{\cdot}05$) suggesting that luteal rescue can occur even at this late stage. In conclusion, desensitization of the CL to hCG appears to be governed by the dose/period for which it is exposed to hCG/dghCG. That desensitization is due to receptor occupancy is brought out by the fact that (i) this can be achieved by giving a larger dose of hCG over a 2 day period instead of a lower dose of the hormone for a longer (4 to 10 days) period and (ii) the effect can largely be reproduced by using dghCG instead of hCG to block the receptor sites. It appears that to achieve desensitization to dbcAMP also it is necessary to expose the luteal cell to relatively high dose of hCG for more than 4 days.

Keywords. Primate; corpus luteum; hCG/dghCG; downregulation.

† Corresponding author (Fax, 91-80-3341683; Email, prl@prl.iisc.ernet.in).

1. Introduction

The luteal phase of non-human primates reckoned from the day of oestrogen/LH surge extends from 12 to 16 days depending upon the species (Moudgal 1984). The corpus luteum (CL) besides producing progesterone (P), synthesizes substantial quantities of oestrogen (Stouffer 1988), relaxin (Weiss *et al* 1976) and inhibin (McLachlan *et al* 1987). Although the CL in the primate is functional for only two weeks in the non-fertile cycle, its life span is extended on pregnancy establishment. It is widely accepted that chorionic gonadotrophin (CG) initially secreted by the pre-attachment blastocyst (Fishel *et al* 1984; Hearn *et al* 1988) and later on implantation of the embryo, by the syncitiotrophoblast (Tullner 1977) is responsible for rescuing the CL from its cyclical imminent death (Knobil 1973).

In a fertile cycle despite a progressive rise in circulating CG, the increment in P production by the CL appears to be only short lived, P levels declining at time points when CG concentration has reached a maximum. Wilks and Noble (1983) and Ottobre and Stouffer (1984) were able to mimic the steroid profile of the fertile cycle in non-mated cycling macaques by administering increasing doses of hCG during the luteal phase. The luteal cells isolated from the pregnancy simulated animals showed desensitization of the adenylate cyclase system to hCG but not to heterologous ligands like prostaglandins, PGE2 and PG12 (Vande Voort *et al* 1988). All the same Ottobre *et al* (1989) observe that desensitization to gonadotrophin in terms of P production could also involve an event subsequent to stimulation of adenylate cyclase. In the pregnancy simulation model used by earlier workers though hCG was administered for 10 days the responsiveness of the luteal cells isolated from these animals at the termination of the experiment were compared only to cells from day 0 of treatment but not to that of cells taken from age matched controls (Vande Voort *et al* 1988; Ottobre *et al* 1989). Such type of comparison does not appear to be correct as it has been clearly demonstrated that the gonadotrophin induced P production by the luteal cell is a function of the age of the CL (Stouffer *et al* 1977; Mukku and Moudgal 1979). Hence, in the present study bonnet monkeys were administered hCG for a 10, 4 or 2 day period (the treatment being initiated either during early or late luteal phase) and the responsiveness of the luteal cells isolated from these animals were compared with that of luteal cells isolated from proper age/day match controls. In addition the ability of dghCG, a well characterized antagonist of hCG, when administered *in vitro* to bring about luteal cell desensitization *in vitro* has also been investigated.

2. Materials and methods

2.1 *Materials*

hCG (CR127 14, 900 units/mg) used for *in vitro* incubations of the luteal cells was provided by Dr G Bialy, NICHD, Bethesda, USA. The deglycosylated hCG (S 2112) was a product of Dr Sairam's laboratory in Montreal. In the LH/CG receptor assay (Selvaraj and Moudgal 1993) it had an activity equivalent to 12,000 units of hCG/mg. In the *in vitro* monkey luteal cell assay, however, dghCG even at a maximal concentration of 100 ng/tube did not evoke a significant steroidogenic response (Selvaraj 1993).

Tritiated progesterone (88 Ci/mmol) and oestradiol (OE_2; 90 Ci/mmol) were purchased from Amersham, UK. DMEM was procured from GIBCO, USA. Collagenase, Hepes,

BSA, dbcAMP etc., were from Sigma Chemical Co., St. Louis, USA. All other chemicals used were of analytical grade and procured locally.

2.2 *Animals*

Healthy adult regularly cycling female bonnet monkeys (*Macaca radiata*) of 8-12 years of age were used. The details of animal husbandry, monitoring of cycle, collection of blood samples etc., were as previously described (Ravindranath *et al* 1989). All the animal experiments were cleared by the institutional ethical committee for use of laboratory animals for biomedical research. The day of onset of menstrual bleeding was considered as the day 1 of the cycle.

2.3 *Experimental protocol*

Regularly cycling non-mated monkeys were administered increasing doses of hCG (Profasi, Serono Laboratories, Randolph, USA) once daily for either 4 or 10 days starting from day 6 or 12 of the luteal phase. For the purpose of this study, the day of oestrogen surge (day 8-10 of cycle) was considered as day 0 of the luteal phase. It should be noted that in these monkeys LH surge occurs within 24 h of $0E_2$ surge (Selvaraj and Moudgal 1993). Monkeys received an increasing dose of hCG-15; 30; 45; 90; 180; 360; 720; 1,440; 2,880 and 5,760 IU/day—over a 10 day treatment period; when the short treatment schedule was used only the first four doses were injected. Deglycosylated hCG was administered from day 6 to 9 of luteal phase either at the same dose as hCG (15, 30, 45, 90 IU/day) or at 100 IU hCG equivalent/day. The results of such a treatment were also compared with that obtained following administration of a high dose (600 IU/day) of hCG or dghCG on days 8 and 9 only of luteal phase. Mid ventral laparotomy was performed under ketamine anaesthesia 24 h after the last injection to remove the CL. Corpus luteum of control animals which were administered saline instead of hCG was removed on either day 10 or 16 or 22 of the luteal phase. The CL of the control animals of day 22 were obtained after the break of the cycle signified by menstruation. Blood was collected at 0930h of the day using Vacutainer tubes (Becton-Dickinson, USA), serum separated and stored at — 20°C until used for steroid estimation.

2.4 *Luteal cell digestion and short term incubation*

The CL was excised from the ovary by blunt dissection and placed in DMEM containing 25 mM Hepes, 0·37% $NaHCO_3$ and 0·1 % BSA (pH 7·4). The luteal tissue was washed and subjected to collagenase digestion essentially according to the method standardized earlier in the laboratory for monkey thecal tissue (Selvaraj and Moudgal 1994). In brief the CL was cut into small pieces and digested thrice with collagenase to obtain cell suspension which was washed extensively with the medium to remove collagenase (Stouffer *et al* 1976). The luteal cells in the DMEM medium were pre-incubated at 37°C for 30 min in a shaker water-bath followed by washing and the cells were suspended in a known volume of medium. Cells were counted in a haemocytometer and 5×10^4 viable cells per tube (65-80% viable cells were obtained

as determined by trypan blue exclusion test) were incubated in the presence or absence of hCG (2 and 200 pg/ml) or dibutryl cAMP (2·5, 25 and 100 μM) in a total volume of 0·5 ml in 22 × 75 mm ether washed glass tubes for 3 h at 37°C in a shaker water-bath (60 oscillations per min). A hCG concentration of 200 pg/ml was chosen as it provided maximal response in a pilot study with monkey luteal cells. At the end of incubation, tubes were transfered to a 20°C freezer until assayed for steroids.

2.5 *Hormone assays*

Oestradiol (OE_2) and P assays were carried out as described elsewhere (Ravindranath *et al* 1989). The serum samples were ether extracted twice and following evaporation of the ether the solids were dissolved in gelatin phosphate buffered saline (GPBS). The *in vitro* incubates were assayed for P without any processing. Progesterone antisera used was a kind gift of Dr Chandana Das, All India Institute of Medical Sciences, New Delhi and the OE_2, antibody was a laboratory preparation. Characteristics of the P and OE_2 antisera used have been provided earlier by Selvaraj and Moudgal (1994).

2.6 *Statistics*

Individual experiments were carried out using 3 monkeys per group and the data presented are mean ± SEM of replicate experiments. In the *in vivo* studies to determine the steroid output over a specified period of time, the area under the curve (summation of steroid concentration) of individual monkeys was computed and analysed using a GP2 software. Differences between the serum oestradiol and progesterone profile of control and experimental animals was statistically evaluated by ANOVA followed by Schefee *F* test using Macintosh based State view 512 + software developed by Brain Power Inc., Ca, USA. In the *in vitro* incubation study the response to each level of hormone addition was determined in triplicate and as the cells from each of the monkeys were analysed separately at each level of hormone the data represents mean of 9 determinations. Treatment differences were analysed by Student's *t*-test.

3. Results

3.1 *Effect of hCG/dghCG administration during luteal phase on serum steroid levels*

Administration of hCG to the cycling monkey for four days during the early luteal phase (day 6-9) resulted in an increase in both serum P (by 78%, $P < 0{\cdot}05$) and OE_2 (by 20%) over the control (figure 1, b vs a and table 1). However, when dghCG was injected at a dose level equal to that of hCG (viz., at 15, 30, 45 and 90 IU hCG equiv/day) from days 6 to 9 of the luteal phase there was no significant change in either P or OE_2 (figure 1, c vs a and table 1). Administration of 100 IU/day dghCG over the same 4 day period (days 6-9 of luteal phase) resulted in reduction in serum P (by 55%, $P < 0{\cdot}05$) levels over the controls (figure 1, d vs a and table 1). Injection of 600 IU hCG on days 8 and 9 of luteal phase brought about a 46% ($P < 0{\cdot}05$) increase in serum P level over the control. The same dose of dghCG did not bring about a change in total P production over the control (figure 1, e and f and table 1). Though the first injection of dghCG

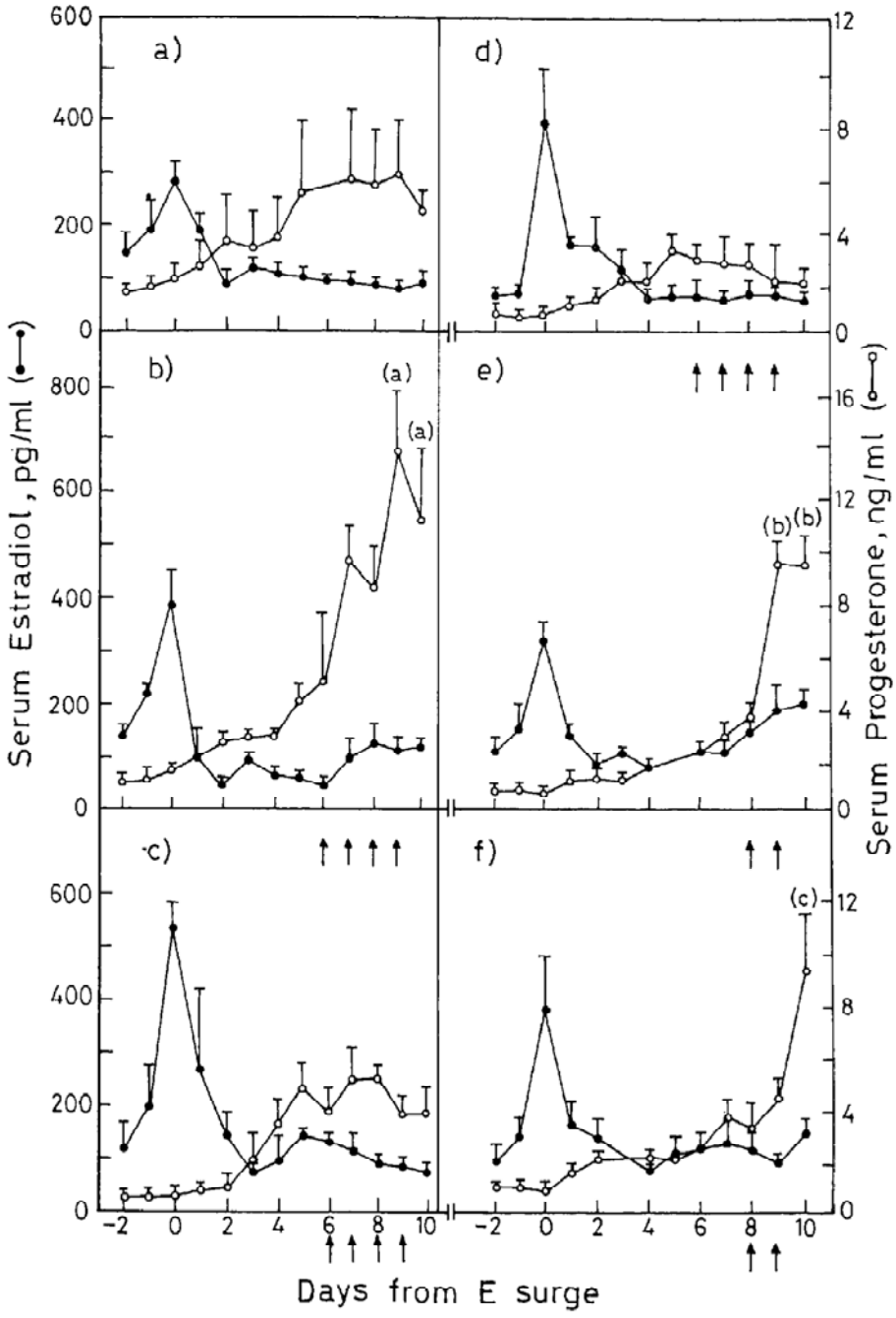

Figure 1. Effect of administration of hCG/dghCG for 4 days (from day 6-9) or for 2 days (on days 8 and 9) of the luteal phase on serum steroid level in female bonnet monkeys. (**a**) Control; (**b**) hCG administered on days 6-9 of luteal phase (15, 30, 45 and 90 IU/day); (**c**) dghCG administration as in **b**; (**d**) dghCG on days 6-9 luteal phase (100 IU/day); (**e**) hCG on days 8 and 9 of luteal phae (600 IU/day) and (**f**) dghCG on days 8 and 9 (600 IU/day). Arrows indicate the days of hormone treatment.

*$P < 0{\cdot}05$ compared to control, (**a**) $P < 0{\cdot}05$ compared to day 5, (b) $P < 0{\cdot}05$ compared to day 8, (c) $P < 0{\cdot}05$ compared to day 8 and 9 (Scheffe F test after ANOVA).

Table 1. Effect of hCG/dghCG injections during the early luteal phase on steroid production.

Treatment			Steroid production Area under the curve (cm²/SEM)* Days 6–10		Days 8–10	
Hormone	Days	Dose (IU)	E	P	E	P
Control	—	—	3·71 ± 0·35	23·07 ± 6·3	1·79 ± 0·25	11·46 ± 3·7
hCG	6–9	15–90	4·47 ± 0·98	41·11 ± 4·8*	—	—
dghCG	6–9	15–90	4·65 ± 0·51	17·65 ± 2·1	—	—
dghCG	6–9	100	2·69 ± 0·16	10·53 ± 1·3	—	—
dghCG	8–9	600	—	—	2·39 ± 0·15	10·27 ± 1·6
hCG	8–9	600	—	—	3·69 ± 0·68	16·90 ± 0·68

Serum steroid concentrations between days 6-10 or 8-10 of the luteal phase (depending on the day of initiation of treatment) was used to calculate the area under the curve using GP2 software.
*$P < 0{\cdot}05$, compared to the control, Student's '*t*' test.
$n = 3$/ group. Data is mean ± SEM.

given on day 8 of luteal phase did not result in an increase in serum P over the basal level the second dghCG injection given on day 9 of luteal phase significantly elevated P levels over the basal ($P < 0{\cdot}05$). The response to second dose is perhaps attributable to the presence of low level of hCG (< 5%) contamination in the dghCG preparation.

Continuing hCG treatment for a 10 day period as per schedule starting from day 6 of luteal phase, resulted in daily increment in P levels. Between days 8-13 there was no significant change in the elevated P levels but beyond this the P level declined despite the animals receiving on the last three days of treatment high doses of hCG (1440, 2880 and 5760 IU). The serum E level in contrast continued to be maintained at an elevated level ($P < 0.05$) (figure 2, a and b and table 2) throughout the experimental period. The fold increment in progesterone produced by hCG treatment ranged between 1·3 to 2·0-fold irrespective of the time of initiation (day 6 vs day 12 of luteal phase) or duration of hCG treatment (for 4 or 10 days) (figure 2b vs c and figure 3b, tables 1 and 2). The net amount of progesterone produced following hCG treatment, however, was higher if the treatment was scheduled between days 6-9 instead of days 12-15 of luteal phase (table 2).

Substantial increase in oestrogen secretion occurred only following hCG treatment for a 10 day period (compare figures 2b and 3b with lb and 2c) and this reached significant proportion (2·6 vs 1·5-fold) when the 10 day treatment was initiated starting from day 12 instead of day 6 of luteal phase (table 2).

3.2 *Effect of hCG/dghCG administration on the weight of the corpus luteum*

The weight of the corpus luteum increased ($P < 0{\cdot}025$) in response to hCG but not dghCG treatment. This was particularly evident when hCG treatment was restricted to a 2 or 4 day period in the early luteal phase (table 3). In a normal cycle the luteal weight declines significantly with age [compare CL wt of day 10 with that of day 22 (table 3)].

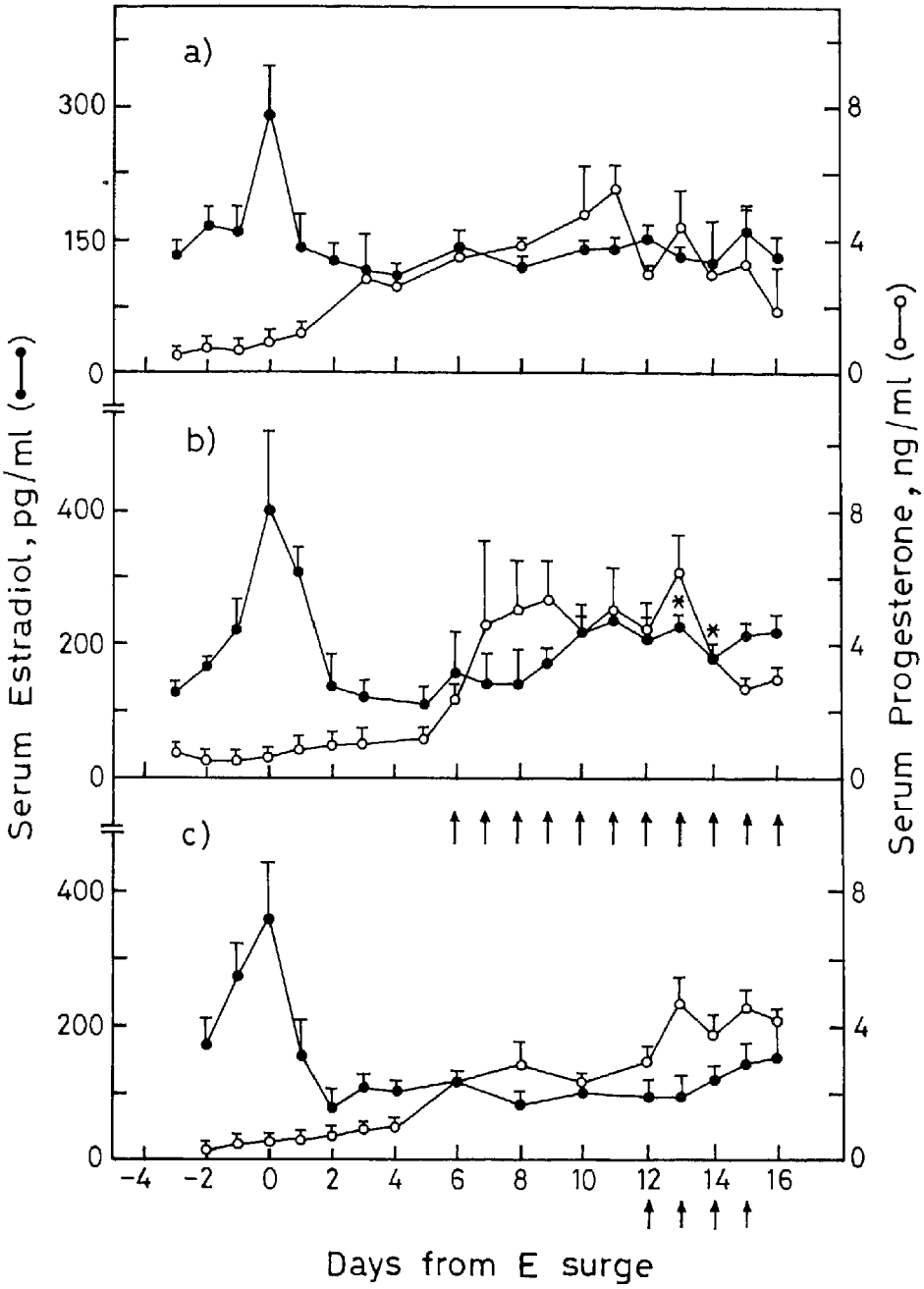

Figure 2. Serum steriod profile of female bonnet monkeys which received different doses of hCG during early (for 10 days) and late (for 4 or 10 days) luteal phase. (**a**) control; (**b**) hCG administered on days 6-15 of luteal phase (15, 30, 45, 90, 180, 360, 720, 1440, 2880 and 5760 IU/day) and (**c**) hCG administered on days 12-15 of the luteal phase with the first four doses of hCG as of **b**. *$P < 0{\cdot}05$ compared to control (ANOVA followed by Scheffe F test).

Table 2. Effect of increasing dose of hCG administration for a 10 day period during early (days 6-16) and late (days 12-22) luteal phase on serum steroid production.

Treatment		Area under the curve (cm^2)			
		Days 6–16		Days 12–22†	
Days	hCG Dose (IU)	E	P	E	P
Control	—	13·24 ± 0·6	39·27 ± 7·0	9·41 ± 0·6	18·2 ± 3·6
6–15	15–5760	19·30 ± 0·8	52·30 ± 8·1*	—	—
12–15	15–90	—	—	—	—
12–21	15–5760	—	—	24·85 ± 5·4**	36·33 ± 5·0*

Total oestrogen and progesterone produced during the period of treatment was determined by calculating the area under the curve using GP2 software. The data presented is mean ± SEM of 3 animals per group.
*P <0·05; **P <0·025 compared to the control, Student's 't' test.
†For the control animals the area under the curve was calculated till day 20 of the cycle.

This decline in CL weight was prevented following hCG treatment for a 10 day period starting from day 12 of luteal phase, a typical result perhaps of luteal rescue.

3.3 *Short term incubation studies with the isolated luteal cells*

Though maximal basal P production (incubated control) by luteal cells from control animals was observed for cells isolated from day 16 of the luteal phase (ng progesterone produced by 5×10^4 cells/3h—day 10: 1·88 ± 0·28 vs day 16: 3·52 ± 0·28 and day 22: 1·42 ± 0·12), the responsiveness to added hCG/dbcAMP in vitro was maximal for luteal cells isolated from day 10(3-4-fold), whereas those from day 16 and 22 showed no increment in P production (tables 4 and 5). The basal secretion of progesterone by the luteal cells isolated from animals treated with hCG *in vivo* during the early luteal phase (from days 6-9 or on days 8 and 9) was significantly increased (10-fold, P <0·005) compared to cells from animals which received saline alone (table 4). The luteal cells of animals treated with high doses of dghCG (600 IU/day) on day 8 and 9 also exhibited elevated basal secretion of progesterone (table 4). The luteal cell preparations which showed high basal secretion of progesterone failed to respond to hCG added *in vitro* (table4). Their ability to respond to dbcAMP, however, appeared not altered (table 4).

Compared to the good response (3·5-fold) the luteal cells (isolated on day 10 of luteal phase) from control animals showed to added hCG (200 pg) the cells from animals treated with low and medium doses of dghCG *in vivo* showed only a 2·0 and 1·7-fold increment in P production respectively (table 4). Relatively the luteal cells from animals treated with high dose of dghCG (600 IU/day × 2) *in vivo*, did not respond to added hCG *in vitro*. In the dghCG treated groups the responsiveness to dbcAMP, however, remained unaltered from the control (table 4). The desensitization obtained with low and medium doses of *in vivo* dghCG treatment becomes significant as at these doses there was no significant increase in *in vitro* basal progesterone production.

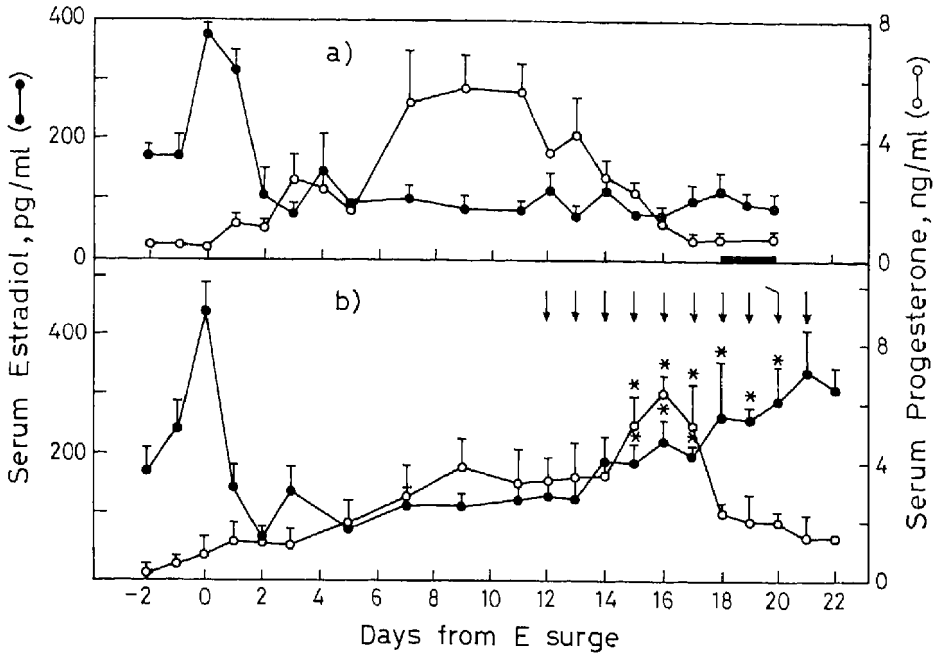

Figure 3. Serum steroid profile of female bonnet monkeys administered hCG from the late luteal phase. (**a**) control (refers to day of menses) and (**b**) KG administered from days 12-21 of the luteal phase (15, 30, 45, 90, 180, 360, 720, 1440, 2880 and 5760 I U/day). *$P < 0{\cdot}05$ (Scheffe *F* lest after ANOVA).

Treatment with hCG for a 10 day period starting from day 6 of luteal phase resulted in total desensitization of the day 16 CL as the cells isolated from such corpora lutea failed to respond to both added hCG and dbcAMP. Though the day 16 luteal cells of control monkeys did not show a dose dependent increase in P production to added hCG and dbcAMP (table 5), the cells of hCG treated monkeys in comparison showed a uniform 30% reduction in basal P output *in vitro* (table 5). Compared to the non-responsive state of the day 22 CL of control monkeys, the cells from the *in vivo* hCG treated group (day 12-21) did respond to the *in vitro* addition of 200 pg/ml hCG ($P < 0{\cdot}05$) and 100 μM dbcAMP ($P < 0{\cdot}05$) thus providing another proof of luteal rescue (table 5).

4. Discussion

The objective of the current study is primarily to understand why luteal rescue initiated during a fertile cycle is not sustained despite continuing increase in CG level. The ability of exogenous hCG to rescue CL from an iminent cyclical death has been shown in the current study by demonstrating that hCG injection during the luteal phase of the non-fertile cycle of the monkey results not only in maintenance of serum P at a heightened level for a longer duration but also in an increase in luteal cell P output *in vitro* (incubated controls) and an increase in overall luteal weight. The overall trophic effect, however, appeared dependent on the time hCG treatment is initiated as well as the dose and duration of the treatment. We have also observed that progesterone production is maintained at a higher level for a longer period ($\approx$ 8 days) if hCG treatment is initiated in the early luteal phase and this essentially confirms the earlier

Table 3. Effect of hCG/dghCG administration during the luteal phase on the weight of the corpus luteum.

Treatment	Days of the treatment	Dose (IU)	Luteal weights (mean ± SEM) Day of laparotomy (luteal phase) Day 10	Day 16	Day 22
Control	Nil	—	69·0 ± 5·3	79·0 ± 14·1	31·0 ± 1·0
hCG	6–9	15–90	92·6 ± 4·7**	—	—
dghCG	6–9	15–90	65·0 ± 1·4	—	—
dghCG	6–9	100	72·0 ± 10·6	—	—
hCG	8,9	600	97·0 ± 3·0*	—	—
dghCG	8,9	600	78·6 ± 8·6	—	—
hCG	6–15	15–5,760	—	71·3 ± 6·9	—
hCG	12–15	15–90	—	91·3 ± 0·54	—
hCG	12–21	15–5,760	—	—	72·0 ± 19·7*

Note that day 10, 16 and 22 of lutel phase represent days 20, 26 and 32 of cycle.
$n = 3$ in each group.
*$P < 0{\cdot}025$; **$P < 0{\cdot}001$ compared to the day matched control.

observation of Ottobre and Stouffer (1984) in the rhesus monkey and that of Ravindranath (1988) in the bonnet monkey.

In the current study we have employed dghCG, a well characterized hCG antagonist to better understand how luteal desensitization to continuous hCG stimulus is affected. While administration of low doses (15-90 IU) of dghCG between days 6-9 of luteal phase did not result in any change in total progesterone produced, injection of 100 IU of dghCG over the same period did bring about a marked reduction (by 54%, $P < 0{\cdot}05$) in progesterone production compared to the untreated controls (table 1). Though the circulating half-life of dghCG is known to be low it is reported to stay bound to the receptor for a much longer time compared to hCG (Sairam *et al* 1985). It is possible such prolonged occupancy of the receptor by dghCG is responsible for block in response not only to endogenous LH but also to hCG addition during *in vitro* incubation. Unlike hCG which showed a consistent ability to increase luteal weight over age matched controls (an expression of trophic effect), dghCG interestingly, at all three doses tried did not bring about an increase in luteal weight (table 2). The increment in luteal weight, it is felt, should be viewed as a true expression of luteotropic activity. The significance of this is brought out better when one considers that ten day hCG injection regimen starting from the late luteal phase prevented the normal decline in the luteal weight seen towards the end of cycle in the control group (table 3).

Earlier workers have demonstrated that responsiveness of the CL to added hCG *in vitro* varies with the age of the CL (Stouffer *et al* 1977; Mukku and Moudgal 1979; Wilks and Noble 1983). The current study has clearly shown that the ability of luteal cell to synthesize progesterone *in vitro* in the absence of added gonadotrophin is maximal for luteal cells of day 16 of luteal phase whereas with added hCG or dbcAMP, the maximal response is exhibited by the young day 10 luteal cells. This suggests the presence of an inverse relationship between *in vitro* basal progesterone production rate and responsiveness to added LH/hCG. As the total receptor concentration of the luteal cell has been observed to remain unchanged during the luteal phase (Ottobre and Stouffer 1986), response to exogenous gonadotropin appears to be determined by the availability of 'free receptors' at

Table 4. Progesterone production *in vitro* by isolated luteal cells on day 10 of the luteal phase from animals exposed to hCG/dghCG treatment *in vivo* for different period.

In vivo treatment		I	II	III	IV	V	VI
Hormone		Nil	hCG	hCG	dghCG	dghCG	dghCG
Days of treatment		Nil	6–9	8, 9	6–9	6–9	8, 9
Dose (IU)		Nil	15–90	600	15–90	100	600
In vitro additions							
Hormone	Dose	Progesterone secreted (ng/5 × 10^4 cells/ml/3 h)					
Nil	Nil	1·88 ± 0·28	18·8 ± 2·1	23·0 ± 4·6	2·80 ± 0·7	2·18 ± 0·28	19·0 ± 0·4
hCG (pg/ml)	2	3·98 ± 0·18**	17·4 ± 1·3	22·4 ± 4·6	4·0 ± 0·78*	3·28 ± 0·38*	13·4 ± 3·4
	200	6·56 ± 0·46**	17·8 ± 1·4	25·2 ± 6·2	5·74 ± 0·50*	3·70 ± 2·20*	18·4 ± 2·4
dbcAMP (μM)							
	2·5	1·6 ± 0·12	17·4 ± 1·46	32·2 ± 4·4**	2·66 ± 0·60	1·68 ± 0·12	24·6 ± 4·0*
	25	2·48 ± 0·3**	19·0 ± 2·58	44·4 ± 6·2**	4·64 ± 0·62*	3·70 ± 0·40*	26·2 ± 2·8*
	100	5·72 ± 0·26**	30·2 ± 4·80*	56·0 ± 6.6**	5·44 ± 1·06*	5·82 ± 0·62*	37·2 ± 3·2*

For further details see § 2.

*$P < 0·05$; **$P < 0·05$, compared to their respective incubated control.

Mean ± SEM (luteal cells from each of the three monkeys were incubated separately in triplicate $n = 9$).

Table 5. *In vitro* responsiveness of luteal cells isolated on day 16/22 of the luteal phase.

In vivo treatment		I	II	III	IV	V
Hormone		Nil	hCG	hCG	Nil	hCG
Days of treatment		Nil	6–15	12–15	Nil	12–21
In vitro additions						
Hormone	Dose	Progesterone secreted (ng/5 × 10^4 cells/ml)				
Nil	Nil	3·52 ± 0·28	2·44 ± 0·12	4·46 ± 0·78	1·42 ± 0·12	3·66 ± 0·36
hCG (pg/ml)	1	3·88 ± 0·24	2·96 ± 0·10	6·72 ± 1·18	1·74 ± 0·12	4·10 ± 0·40*
	200	4·20 ± 0·84	2·98 ± 0·18	4·98 ± 0·80	1·54 ± 0·18	4·46 ± 0·24*
dbcAMP (μM)						
	2·5	3·26 ± 0·34	2·66 ± 0·26	4·86 ± 0·80	1·74 ± 0·18	3·48 ± 0·42
	25	2·90 ± 0·34	2·64 ± 0·59	4·50 ± 0·40	1·88 ± 0·12	3·58 ± 0·54
	100	3·48 ± 0·62	2·98 ± 0·56	5·02 ± 1·06	1·66 ± 0·22	5·00 ± 1·14*

hCG was administered increasing doses-15, 30, 45, 90, 180, 360, 720, 1440, 2880 and 5760 IU/day either from day 6/12 of luteal phase for 4/10 days and the luteal cells obtained from the corpus luteum of these animals on either day 16 (group I-III) or 22 (groups IV and V) of the luteal phase were checked for their responsiveness as described in the text.

*$P < 0·05$ compared to the respective incubated control.

Mean ± SEM ($n = 3$ in each group).

any given time and this seems to be a function of the period and/or the dose of LH/CG the luteal cell is pre-exposed to. It is to be presumed that the luteal cells of day 16 CL relative to that of day 10 CL exhibit a greater degree of LH receptor occupancy by endogenous LH and consequently are less responsive to added CG *in vitro*. Cameron and Stouffer (1982) have shown that the binding affinity of LH or hCG to the luteal cell receptor is essentially similar and Ottobre and Stouffer (1986) have reported that receptor occupancy increase with exogenous hCG treatment.

The observation that luteal cells of monkeys treated with increasing doses of hCG *in tiro* respond to addition of dbcAMP but not hCG in vitro clearly indicates that desensitization is affected by interfering with adenylate cyclase activation leading to cAMP production. The use of dghCG, an antagonist capable of binding hCG receptor but not eliciting a response, in the current study permits us to infer that desensitization is primarily achieved due to greater than normal occupancy of the receptor by the hCG/dghCG injected. It is possible that the endogenous LH load on the receptor also increases with the age of the CL. The fact that the luteal cells taken on day 16 of luteal phase produce greater amounts of basal progesterone compared to that of day 10 of luteal phase (day 16: 3·52 ± 0·28 vs day 10:1·88 ± 0·28 ng progesterone) perhaps is a reflection of this phenomenon and appears to be inversely related to the response to LH added *in vitro*, increment in P secretion effected by addition of 200 pg hCG being 3·5-fold for day 10 compared to 1·2-fold for day 16 CL. By giving exogenous hCG or dghCG *in vitro* we could be precipitating the loss of responsivity to added hCG *in vivo* (desensitization) and this could be consequence of increase in the rate/extent of receptor occupancy by the *in vivo* CG treatment. This becomes particularly evident when one examines the *in vitro* responsiveness of luteal cells of animals treated with dghCG (100 IU) *in vivo* to addition of hCG (200 pg/ml). The fold increment in P secretion was reduced by 50% (fold increment control 3·5 vs dghCG 1·7, $P < 0·05$).

It is evident from the current study that while the luteal cell of normal day 16 luteal phase had lost its responsiveness to dbcAMP, the day 10 luteal cell pre-exposed to hCG for only

2 or 4 days still retained its ability to respond to dbcAMP. However, desensitization to dbcAMP could be achieved by prolonging (by 10 days) the exposure of the luteal tissue to hCG action *in vivo* suggesting thereby that desensitization at the post cAMP stage is a secondary effect.

The reason why continuous hCG administration results in decline in P but not OE_2 production from the luteal tissue has been puzzling investigators. A recent study of Benyo *et al* (1993) has shown that there was a dramatic increase in mRNA levels for the aromatase enzyme and low density lipoprotein receptor in luteal tissue of animals given hCG both in early and late luteal phase. The difference we observe in P and E secretory pattern following hCG stimulus *in vivo* could be explained if we assume that the granulosa lutein and thecal lutein cells of the CL have differential sensitivity to hCG desensitization. Our recent studies have shown that while the *in vitro* responsiveness of granulosa cells aspirated from follicles of monkeys specifically deprived of LH support for 48 h (by LH antibody treatment), to added hCG was significantly inhibited, the thecal cell responsivity to added hCG *in vitro* remained unaltered (Selvaraj and Moudgal 1994). These results suggested that there are differences in the sensitivity of the follicular thecal and granulosa cell LH receptor mechanism to up regulation and we now postulate that this could be true for down regulation of lutenized thecal and granulosa cells also. Since the thecal cells are known to produce oestrogen (Channing 1980) it is probable that the lack of reduction in E output following continuous hCG treatment is due to these cells not being desen-sitized by hCG treatment. This can only be verified if the luteal cell derived from the two cell types are examined separately for susceptibility to desensitization. In support of this is the observation of Marut *et al* (1983) that following aspiration of granulosa cells from the preovulatory follicle, progesterone, but not oestrogen secretion declines.

It appears from the current study that desensitization obtained by exposing luteal cells to hCG for short periods is perhaps restricted to the first event in hormone action viz. adenylate cyclase activation and cAMP production. These desensitized cells unlike those exposed to hCG action for long durations (for 10 days or normal day 16 of luteal phase) continue to be responsive to cAMP. The current study shows that desensitization can be achieved by exposing the luteal tissue *in vivo* not necessarily to a long duration of hCG treatment but to a relatively higher concentration of hCG for shorter durations. Secondly, by using dghCG as the ligand it has been possible to demonstrate without bringing about any increment in *in vivo* P production that there is a correlation between threshold of receptor occupancy and desensitization.

Acknowledgements

Our thanks are due to Ms K Vijayalakshmi and Mrs Srilatha for the technical help. This work was aided by grants from the Indian Council of Medical Research and Indian National Science Academy, New Delhi.

References

Benyo D F,Little-Ihrig I and Zeleznik A J 1993 Nonco-ordinated expression of luteal cell messenger ribonucleic acids during human chorionic gonadotropin stimulation of the primate corpus luteum; *Endocrinology* **133** 699-704

Cameron J L and Stouffer R L 1982 Gonadotropin receptors of the primate corpus luteum II. Changes in available luteinizing hormone and chorionic gonadotropin-binding sites in macaque luteal membranes during the non-fertile menstrual cycle; *Endocrinology* **110** 2068-2073

Channing C P 1980 Progesterone and estrogen secretion by cultured monkey ovarian cell types: Influences of follicular size, serum luteinizing hormone levels, and follicular fluid estrogen levels; *Endocrinology* **107** 342-352

Fishel S B, Edwards R G and Evans C J 1984 Human chorionic gonadotropin secreted by preimplantation embryos cultured *in vitro*; *Science* **223** 816-818

Hearn J P, Hodges J K and Gems S 1988 Early secretion of chorionic gonadotrophin by marmoset embryos *in vivo* and *in vitro; J. Endocrinol*. **119** 249-255

Knobil E 1973 On the regulation of primate corpus luteum; *Biol. Reprod*. **8** 246-258

Marut E L, Huang S C and Hodgen G D 1983 Distinguishing the steroidogenic role of granulosa and thecal cells of the dominant ovarin follicle and corpus luteum; *J. Clin. Endocrinol. Metab*. **57** 925-930

McLachlan R I, Robertson D M, Healy D L, Burger H G and de Krester D M 1987 Circulating immunoreactive inhibin levels during the normal menstrual cycle; *J. Clin. Endocrinol. Metab*. **65** 954-961

Moudgal N R 1984 Corpus luteum of the non-human primate; *Adv. Vet. Sci. Comp. Med*. **28** 343-366

Mukku V R and Moudgal N R 1979 Regulation of Corpus luteum function in the non human primate- a study in bonnet monkeys; in *Recent advances in reproduction and regulation of fertility* (ed.) G P Talwar (North Holland: Elsevier) pp 135-142

Ottobre J S, Houmard B S and Ottobre A C 1989 Luteal production of steroids and prostaglandins during simulated early pregnancy in the primate: Differential regulation of steroid production by choriomc gonadotropin; *Biol. Reprod*. **41** 393-400

Ottobre J S and Stouffer R L 1984 Persistent versus transient stimulation of the macaque corpus luteum during prolonged exposure to human chorionic gonadotropin: A function of age of the corpus luteam: *Endocrinology* **114** 2175-2182

Ottobre J S and Stouffer R L 1986 Receptors for chorionic gonadotropin in the corpus luteum of rhesus during simulated early pregnancy: lack of down-regulation; *Endocrinology* **119** 1594-1602

Ravindranath N 1988 *Studies on the reproductive endocrinology of the female bonnet monkey* (*Macaca radiata): Hormonal regulation of follicular maturation, luteal function and implantation,* PhD Thesis, Indian Institute of Science, Bangalore

Ravindranath N, Sheela Rani C S, Martin F and Moudgal N R 1989 Effect of FSH deprivation at specific times in follicular maturation in the bonnet monkey (*Macaca radiata*); *J. Reprod. Fertil*. **87** 231-241

Sairam M R 1985 Protein glycosylation and receptor-ligand interactions; in *The receptors* (ed.) M P Conn (New York: Academic Press) vol. 2, pp 307-340

Selvaraj N 1993 Studies on regulation of follicular maturation, luteal function and implantation in female bonnet monkey (*Macaca radiata*), Ph.D. Thesis, India, Indian Institute of Science, Bangalore

Selvaraj N and Moudgal N R 1993 Development of an LH receptor assay capable of measuring serum LH/CG in a wide variety of species; *J. Reprod. Fertil*. **98** 611-616

Selvaraj N and Moudgal N R 1994 *In vivo* and *In vitro* studies on the differential role of luteinizing hormone and follicle stimulating hormone in regulating follicular function in the bonnet monkey (*M. radiata*) using specific gonadotropin antibodies; *Biol. Reprod*. **51** 246-253

Stouffer R L 1988 Perspectives on the corpus luteum of the menstrual cycle and early pregnancy; *Semin Reprod. Endocrinol*. **6** 103-113

Stouffer R L, Gulyas B J, Nixon W E and Hoden G D 1977 Gonadotropin sensitive progesterone production by rhesus monkey luteal cells *in vitro*: A function of age of the corpus luteum during the menstrual cycle: *Endocrinology* **100** 506-512

Stouffer R L, Nixon W E, Gulyas B J, Johnson D K and Hodges G D 1976 *In vitro* evaluation of corpus luteum function of cycling and pregnant rhesus monkeys: Progesterone production by dispersel luteal cells; *Steroids* **27** 543-551

Tullner W W 1977 Comparative aspects of primate chorionic gonadotropins; in *Contribution to primatology* (ed.) H Kuhn (Basel: S Karger) vol. 3, pp 235-257

Vande Voort C A, Molskness T A and Stouffer R L 1988 Adenylate cyclase in the primate corpus luteum during chorionic gonadotropin treatment simulating early pregnancy: Homologous verses heterologous desensitization; *Endocrinology* **122** 734-740

Weiss G, O'Byrne E M and Steinetz B G 1976 A product of the human corpus luteum of pregnancy; *Science* **194** 948-949

Wilks J W and Noble A S 1983 Steroidogenic responsiveness of monkey corpus luteum to exogenous gonadotropin; *Endocrinology* **112** 1256-1266

Corresponding editor: SAMIR BHATTACHARYA