

Adenylyl Cyclase Activities in Ovarian Tissues. IV. Gonadotrophin-Induced Desensitization of the Luteal Adenylyl Cyclase Throughout Pregnancy and Pseudopregnancy in the Rabbit and the Rat

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ABSTRACT. We measured the adenylyl cyclase (AC) activity in dissected CL and the responsiveness of the AC system to LH, FSH, and prostaglandin (PG)E₁ at different times following the administration of high doses of hCG or hLH to pseudopregnant and pregnant rats and rabbits.

In rabbits, ovulatory doses of hCG promoted desensitization of the AC system in both CL of pregnancy and CL of pseudopregnancy (PSP), but at varying rates. At least a 50% decline in the LH-stimulated AC system was demonstrable 2 h after the hCG injection in CL obtained during PSP and the first 18 days of pregnancy. However, after day 21, AC activity was unaltered at 2 or 24 h after hCG injection, necessitating as much as 72 h for the AC system to become desensitized to LH. It seems that CL in the last third of pregnancy are afforded partial protection from the desensitizing effects of hCG. This protective effect was found not to be conferred upon follicles contained in ovaries after day 21 of pregnancy or upon newly, hCG-induced 3-day-old CL in 24-day pregnant rabbit ovaries.

hCG-induced desensitization of CL adenylyl cyclase in rabbits was prevented neither by cauteriza-

tion of tertiary follicles nor by the continued administration of estradiol-17 β (1.5 μ g sc twice daily), suggesting that this effect of hCG is due to a direct interaction with the CL, and not due to interruption of the follicular estrogen supply.

In rats, the injection of an ovulatory dose of hCG (50 IU sc into prepubertal rats; 50 IU ip plus 50 IU sc into mature rats) also induced desensitization of the AC system in ovaries of superovulated prepubertal rats and in CL of pseudopregnant and pregnant rats. Desensitization of the AC system was not detectable at 2 h, was 30% of total by 6 h, and was complete at 24 h after hCG injection.

Both regression of the CL and desensitization of the AC system in CL are induced only by doses of hCG which are ovulatory and not subovulatory. Desensitization of AC appears to precede functional luteolysis, at least in the pseudopregnant rabbit. Thus, the apparent close association between hCG-induced luteolysis and the desensitization of the adenylyl cyclase system in CL would suggest that desensitization may be a marker for luteal regression. (*Endocrinology* 99: 211, 1976)

LH AND HUMAN (h)CG can exert either luteotrophic or luteolytic effects in rats and rabbits, depending on the dosage of gonadotrophins administered. Doses of LH or hCG which induce the ovulation of mature Graafian follicles also induce luteolysis in either pregnant or pseudopregnant animals, and subovulatory doses induce

neither ovulation nor luteolysis (1,2). However, even though it can occur during pregnancy and pseudopregnancy (PSP), ovulation *per se* is not a necessary prerequisite for luteal regression (2,3). In rabbits, ovulatory doses of hCG or LH can induce luteal regression throughout pregnancy and PSP, except on day 2 after ovulation (1,4,5). In rats, luteal regression has been reported following the injection of LH into hypophy-

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sectomized rats bearing autotransplanted pituitaries (2), into animals with prolonged PSP (6) or normal pregnancy (7,8), into hypophysectomized rats with persistent corpora lutea (CL) (9), and into pregnant mare serum gonadotrophin (PMSG)-primed immature rats (3). The above reports are inconsistent with that of Madhwa-Raj and Moudgal (10), who reported no luteal involution in pregnant rats following injections of LH which induced ovulation and a second set of CL.

Because of our findings that ovulatory doses of hCG caused desensitization of rabbit follicle adenylyl cyclase (AC), that the ovulation of rat and rabbit follicles was coincident with a loss of responsiveness of the AC system, and that such a loss of responsiveness was also seen in rabbit CL of PSP following the administration of hCG (11,12), and because the desensitizing effect of hCG appeared to be rapid and possibly related to the induction of luteolysis, we investigated the possibility that this effect could be induced also in the CL of pregnant rabbits and in various types of CL of the rat. It will be shown that desensitization appears to be a direct effect on CL (as is stimulation of AC), that it can be induced by hCG in both rabbits and rats, be they pseudopregnant, pregnant, or superovulated, as long as "ovulatory" doses of the gonadotrophin are used, and that the rate of response varies with the type of CL studied. The possible physiologic implications of desensitization of AC in ovarian structures are discussed.

Materials and Methods

Animals

The care and maintenance of cycling, pseudopregnant, and pregnant rats and of estrous, pseudopregnant, and pregnant rabbits have been described previously (11,12). For superovulation studies, rats were received (unless otherwise stated) at 21 days of age (Charles River, CD outbred) and were superovulated by injecting them at 22 or 23 days of age with PMSG (50 IU, sc) and 56 h later with hCG (50 IU, sc). Ovulation

was induced in pregnant and pseudopregnant rabbits with the injection of 100 IU hCG, iv. Ovulation was induced in pregnant and pseudopregnant rats with the injection of 50 IU hCG, ip, plus 50 IU hCG, sc, since 50 IU hCG either ip or sc did not induce ovulation. In rats and rabbits, the day after the induction of ovulation (day of expected estrus in rats) was counted as day 1.

Cauterization of the follicles (FLX) during PSP was performed by anesthetizing the rabbit with pentobarbital (approximately 25 mg/kg, iv), exposing the ovary by a midline incision, and applying a red-hot needle (approximately 1 mm in diameter) to all antral follicles. Following FLX, the muscle layers and skin were sutured. Control FLX animals received no further treatments; experimental FLX animals received 100 IU hCG, iv following suturing. All animals were sacrificed 1 h after suturing was completed.

Materials

PMSG (NIAMDD-PMSG-1) was the gift of the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). hLH (lot no. BIB1) was the gift of Dr. Robert J. Ryan, Department of Molecular Medicine, Mayo School of Medicine, Rochester, Minnesota.

Adenylyl cyclase assay

Adenylyl cyclase activity was determined as described in the two preceding reports (11,12). Unless otherwise stated, all other materials and methods were also as described in the previous 2 reports of this series (11,12).

Results

Desensitization of adenylyl cyclase in corpora lutea during pseudopregnancy and pregnancy in the rabbit

Since LH can directly affect the rabbit CL by stimulating its AC system (11,13,14), we wanted to determine whether the hCG-induced desensitization of AC in the CL of PSP rabbits is a direct effect on the CL, or whether the action is mediated *via* the follicle by inducing ovulation and thus terminating estrogen support for the CL (15,16). Two experiments were carried out to answer

this question. First, we tested whether the removal of follicles would, by itself, lead to desensitization, and assuming that it might not, whether hCG was able to induce desensitization in the absence of follicles, thus testing for a possible follicular contribution to the occurrence of desensitization in CL. As shown in Fig. 1, cauterization of tertiary follicles (responsive to hGH and LH) neither resulted within 1 h in desensitization nor interfered with desensitization induced by hCG. Second, we tested whether estrogen administration would interfere with desensitization by hCG, as seen either after 2 h or after 3 days. As shown in Table 1, the administration of estradiol-17 β (1.5 μ g, twice daily, starting 25 h before hCG injection) did not interfere either with the action of hCG, seen after 2 h, or with the persistence of hCG-induced desensitization, seen after 3 days (for further information on the persistence of the effect of hCG see below). The regimen of estrogen administration used in this experiment has

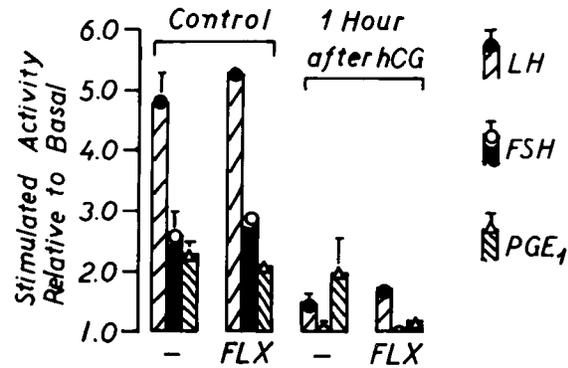


FIG. 1. Lack of involvement of ovarian Graafian follicles in the ability of hCG to induce a loss of responsiveness of luteal adenylyl cyclase to LH. Adenylyl cyclase activities were determined in homogenates of CL obtained from ovaries of 6-day pseudopregnant rabbits whose Graafian follicles had been left intact (-) or removed by cauterization (FLX) 1 h prior to dissection and homogenization. Control indicates rabbits that received no hCG injection. CL from 2 rabbits were used in each assay. The number of these assays were 6 for control CL from ovaries with follicles and for CL from ovaries with follicles from rabbits treated with hCG (100 IU/3.5-4.5 kg rabbit, iv). For other details see *Materials and Methods*.

TABLE 1. Desensitization and persistence of desensitization induced by hCG in corpora lutea of pseudopregnant rabbits receiving estrogen

Time of pseudopregnancy (days)	Treatments	Age of CL tested (days)	Weight of CL tested ² (mg)	Adenylyl cyclase activities ¹		
				Basal	LH-stimulated	(n)
6	None	6	11.3 (16)	38.0 ± 8.0	158.5 ± 7.5	(2)
	hCG ³	6	9.1 (18)	63.5 ± 8.5	82.0 ± 4.0	(2)
	hCG ³ plus E ₂ ⁴	6	11.3 (13)	51.5 ± 7.5	71.0 ± 1.0	(2)
9	None	9	13.2 (24)	25.0 ± 6.0	124.0 ± 16.0	(2)
	hCG ⁵	9	9.3 (23)	22.0 ± 3.0	67.0 ± 2.5	(2)
	hCG ⁵	3	4.9 (14)	20.0 ± 2.0	153.5 ± 0.5	(2)
	hCG ⁵ plus E ₂ ⁶	9	21.0 (34)	34.0 ± 4.0	58.0 ± 3.0	(4)
	hCG ⁵ plus E ₂ ⁶	3	3.3 (25)	30.0 ± 7.5	133.0 ± 18.0	(3)

¹ Values are mean ± SEM of *n* assays in each of which the activities in homogenates of CL from both ovaries of one rabbit were determined in duplicate.

² Mean weight of the number of dissected CL shown in parentheses.

³ 75 IU/3.5-4.5 kg rabbit administered iv at 0900 h of day 6 of pseudopregnancy. Animals were sacrificed at 1100 h of the same day.

⁴ Treatment with estradiol-17 β (E₂) consisted of 3 sc injections of 1.5 μ g each (in 0.1 ml peanut oil) administered at 12 h intervals and started at 0800 h of day 5 of pseudopregnancy.

⁵ 75 IU/3.5-4.5 kg rabbit administered iv at 0900 h of day 6 of pseudopregnancy. Animals were sacrificed at 0900 h of day 9 of pseudopregnancy. Both sets of CL, the original 9-day-old set and the new 3-day-old set (identified by fresh ovulation points and small size), were tested separately for their activities.

⁶ Treatment with estradiol-17 β (E₂) consisted of 9 sc injections of 1.5 μ g each (in 0.1 ml of peanut oil) administered at 12 h intervals and started at 0800 h of day 5 of pseudopregnancy.

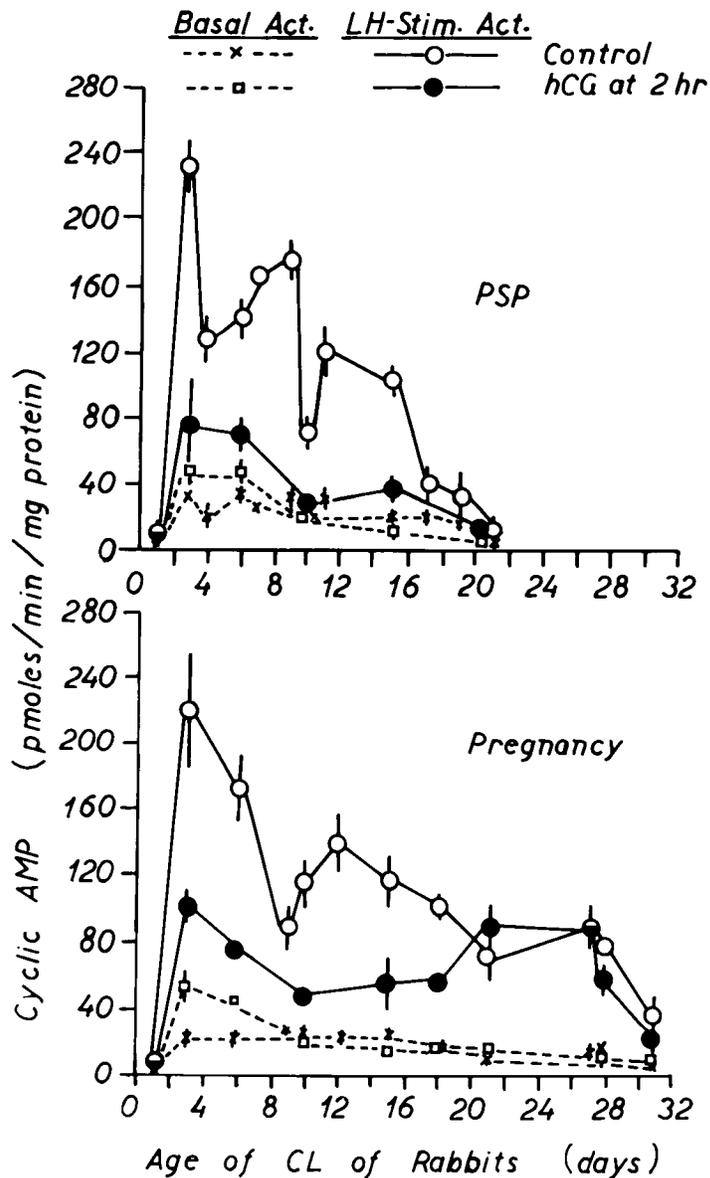


FIG. 2. Effect of a desensitizing dose of hCG (100 IU/3.5–4.5 kg rabbit, iv) injected on various days of PSP (upper panel) or pregnancy (lower panel) on basal and LH-stimulated adenyl cyclase activity in homogenates of CL obtained 2 h after the hCG injection. When present, LH was 10 μ g/ml. Single points represent one assay in which a minimum of 2 rabbits was used. Mean \pm SEM is shown where more than 2 assays were performed. For pseudopregnant rabbits receiving hCG for 2 h on days 3, 6, 10, 15, and 21, *n* equals 2, 6, 2, 2, and 2, respectively. For pregnant rabbits receiving hCG for 2 h on days 2, 15, 21, 28, and 31, *n* equals 2. For all other points, *n* equals 1. Data for control curves are from reference 11.

been shown in several laboratories to be luteotrophic in the rabbit, as evidenced by maintenance of CL weight and progesterone secretion (16–20). Furthermore, the finding that the mean weight of 9-day-old corpora lutea from PSP rabbits receiving estrogen for 4 days was larger than that of CL from animals not receiving estrogen, *i.e.*, (mean \pm SEM in mg) 21.0 ± 1.34 (hCG plus E_2 , *n* = 4) *vs* 9.3 ± 1.1 (hCG alone, *n* = 2), is indicative that sufficient estrogen was ad-

ministered to effect CL size changes, and presumably also to exert a luteotrophic (steroidogenic) effect. The regimen of estrogen administration used in the experiment of Table I also appeared to affect hCG-induced ovulation in PSP rabbits, for of 4 rabbits receiving estrogen treatment, 2 responded to hCG on day 6 with 9 and 12 new ovulations (mean weight of new CL on day 9 was 4.7 and 1.8 mg per CL, respectively), 1 had only 4 new CL by day 9 (4.5 mg per CL)

and 1 did not show any new CL by day 9, even though many 2–3 mm haemorrhagic follicles were observed. The reasons for these variations were not investigated.

We found that desensitization of AC in rabbit CL could be induced at any time during PSP or pregnancy; however, the rate of desensitization varied. Thus, during PSP, the AC system was rapidly desensitized by an ovulatory dose of hCG (100 IU, iv), exhibiting at least a 50% decline in the LH-stimulated AC at 2 h after hCG injection (Fig. 2, upper section). The same was observed during the first 18 days of pregnancy, but not afterwards (Fig. 2, lower section; Fig. 3, upper section). Thus, the AC system in CL obtained from rabbits during the last third of pregnancy did not show desensitization 2 h after hCG, and required as much as 72 h (3 days) to show this effect unequivocally.

We never tried to induce desensitization of CL AC on days 1 or 2 of PSP since the cyclase system is still unresponsive to gonadotrophin stimulation at these times. It is interesting that Spies *et al.* (5) found that CL on day 2 of PSP are resistant to the luteolytic effects of hCG, suggesting that the developing cyclase system might be resistant to ovulatory doses of hCG in its early stages of development.

The injection of an ovulatory dose of hCG into pseudopregnant or pregnant rabbits also caused an increase in CL basal AC activity at 2 h post injection (Table 1, Fig. 2, ref. 11). This was especially noticeable during the first portion of pregnancy or PSP when LH-stimulated AC activities were highest, and suggests an occupation of the receptors coupled to the AC system by the injected hCG. In experiments not shown here, we found that hCG stimulates follicle and CL AC activity *in vitro* to the same extent as does ovine or bovine LH.

The desensitization of AC in CL induced by ovulatory doses of hCG seems to be permanent. We tested LH-stimulated AC in the CL of rabbits, that had received a desensitizing dose of hCG on the tenth day of preg-

nancy and were tested 14 days later (Fig. 3A), and found it to be low, comparable to that determined in interstitial tissue. By this time (14 days after hCG), the original CL had regressed and had become corpora albicantia.

The induction of ovulation by hCG (100 IU, iv) on day 21 of pregnancy produced new CL whose AC activity could be rapidly desensitized by hCG on day 24 of pregnancy (85% decline at 2 h after hCG, Fig. 3B), in spite of that fact that the AC in CL which were formed at mating was considerably more resistant to the desensitizing effects of hCG, requiring 72 h for desensitization to be completed. We observed no fetal death following the induction of ovulation at any of the times tested (see Fig. 3).

Desensitization of adenylyl cyclase in follicles during pseudopregnancy and pregnancy in the rabbit

The injection of an ovulatory dose of hCG into pregnant and pseudopregnant rabbits not only produced desensitization of the luteal AC but also promoted ovulation of the preovulatory follicles. As in preovulatory estrous rabbit follicles (11), the AC in follicles contained in the ovaries of pseudopregnant and pregnant rabbits was desensitized to ovulatory doses of hCG but appeared to become so at a slower rate (Table 2). Thus, while estrous follicles had lost more than 70% of their LH-sensitive AC by 2 h after hCG injection, the follicles of pregnancy and PSP had lost 36 and 45% of their respective LH-sensitive activity.

Adenylyl cyclase activity in corpora lutea induced during pregnancy in the rabbit

Following hCG-induced ovulation during pregnancy in the rabbit, new CL are formed. The levels of LH-stimulated AC activity attained in these hCG-induced CL are shown in Fig. 3B. Twenty-four h after the induction of ovulation by hCG, the AC system in the new CL was unresponsive to LH stimulation, as might be expected by the

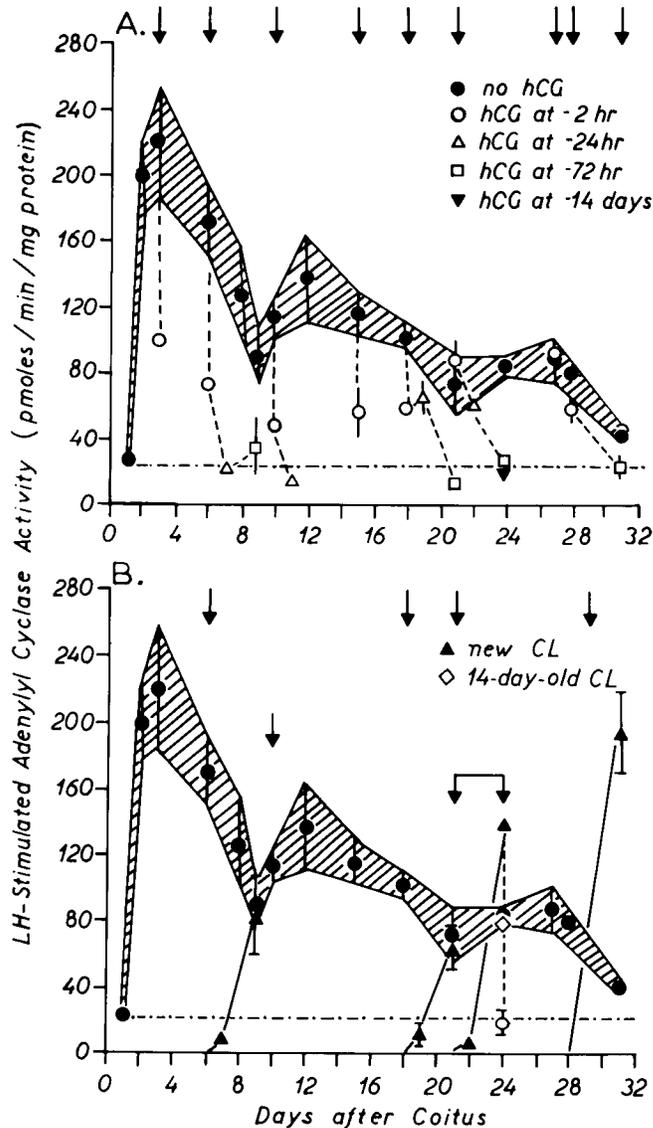


FIG. 3. Time courses of hCG-induced desensitization of adenylyl cyclase to LH stimulation in pregnant rabbits. hCG (100 IU/3.5–4.5 kg rabbit, iv) was injected at the days indicated by the arrows and LH-stimulated adenylyl cyclase activity was then determined in excised CL 2 h (○), 1 day (△), 3 days (□), and 14 days (▼) after hCG injection. ---, represents the time courses for desensitization of LH-stimulated CL adenylyl cyclase. - · - · - ·, represents LH-stimulated activity in interstitial tissue. ●, represents LH-stimulated adenylyl cyclase activity of pregnant control rabbit CL which received no treatment. Each point represents one assay in which a minimum of 2 rabbits were used. Shaded area and vertical bars: SEM of means where 2 to 6 assays were performed. For rabbits receiving hCG injections 2 h prior to the assay on days 3, 15, 21, 28, and 31, *n* equals 2. For rabbits receiving hCG injections 24 h prior to the assay on days 10 and 18, *n* equals 2. For rabbits receiving hCG injections 72 h prior to the assay on days 6 and 28, *n* equals 2. For all other points, *n* equals 1. Control data are from reference 11. B. (same experiment): LH-stimulated adenylyl cyclase activity in newly induced CL in pregnant rabbits and desensitization of the new CL by hCG in late pregnancy. hCG (100 IU/3.5–4.5

kg rabbit, iv) was injected at the days indicated by the arrows, and LH-stimulated adenylyl cyclase activity was determined in newly formed CL 1 and 3 days after the induction of ovulation (▲). In one set of rabbits, a second hCG injection was given (double arrow) and the activity of the newly formed, 3-day-old CL was determined 2 h after the second (desensitizing) hCG injection (○). In another set of pregnant rabbits, hCG was injected on day 10 (lowered arrow) and adenylyl cyclase activity was determined in the new CL 14 days later (on day 24 of pregnancy) (◇). For rest of details see legend to Fig. 3A. Note: LH-stimulated activity in 1-day-old, hCG-induced CL was less than that of interstitial tissue. This is due to the fact that 1-day-old CL do not respond to LH (ca. 1.2-fold stimulation) while interstitial tissue is stimulated about 3-fold (basal and LH-stimulated activity (mean in pmoles/min/mg ± SEM of 4 assays) were 7.2 ± 1.4 and 23.8 ± 5 , respectively). For 1-day-old CL induced with hCG on day 18, for 3-day-old CL induced with hCG on days 6, 18, and 28, and for 3-day-old hCG-induced CL contained in the ovaries of 24-day pregnant rabbits and desensitized for 2 h (○), *n* equals 2. For all other points, *n* equals 1.

equally unresponsive AC system in CL obtained 24 h after the induction of ovulation in estrous rabbits by mating or by an hCG injection (11). By 72 h, however, the degree of stimulation varied with the stage of pregnancy during which ovulation was induced. Thus, when hCG was injected on days 6 or 18, the LH-stimulated AC activities acquired by the new CL at 72 h were equivalent to those of the respective days of pregnancy (days 9 and 21), and were therefore considerably lower than those found in 3-day-old CL obtained from normal PSP rabbits (ovulation induced in estrous rabbits). On the other hand, when hCG was injected in the last third of pregnancy (on days 21 or 28), the LH-stimulated AC activity at 72 h in the new CL was greater than on the respective days of pregnancy (days 24 and 31) and seemed to approach the activity levels of normal 3-day CL, especially in CL induced on day 28 of pregnancy.

When ovulation was induced on day 10 of pregnancy, the LH-stimulated AC activity measured in the 14-day-old CL on day 24 of pregnancy was about 50% as active as that of 14-day-old CL of PSP or pregnancy (in Fig. 3B), and equivalent to that of CL formed at mating in 24-day pregnant rabbits, suggesting that these 14-day-old CL were subject to, as well as responsive to, the luteotrophic controls of 24-day pregnant rabbits.

Desensitization of adenylyl cyclase in corpora lutea during pseudopregnancy and pregnancy in the rat

The injection of ovulatory doses of hCG into pseudopregnant, pregnant, and superovulated (PMSG-primed) rats resulted in desensitization of the AC system in CL. When hCG (50 IU, ip, plus 50 IU, sc) was injected into pseudopregnant rats on days 5, or 9, no changes in luteal LH-stimulated AC activity were detected at 2 h after hCG injection; however, there were large increases in basal activity (Fig. 4), again presumably due to

TABLE 2. Effect of hCG injection on adenylyl cyclase activities in homogenates of follicles larger than 2 mm in diameter from ovaries of pregnant and pseudopregnant rabbits

Treatment ^a	Adenylyl cyclase activities ^b	
	Basal	LH-stimulated
Estrous follicles:		
None (n = 5) ^c	8.3 ± 3.8	70.2 ± 11.9
hCG at -2 h (n = 3)	12.2 ± 5.1	15.6 ± 2.4
Follicles of pregnancy: ^d		
None (n = 16)	3.6 ± 0.4	26.7 ± 2.5
hCG at -2 h (n = 10)	8.8 ± 0.9	17.7 ± 1.6
Follicles of pseudopregnancy: ^e		
None (n = 11)	3.7 ± 0.4	29.9 ± 3.6
hCG at -2 h (n = 10)	6.5 ± 0.8	15.9 ± 1.0

^a When indicated, 100 IU hCG was injected iv per 3-4.5 kg rabbit 2 h prior to sacrifice.

^b Values represent pmoles cAMP formed per min per mg homogenate protein ± standard deviation of the mean. When added to the assay, LH (NIH-LH-B9) was 10 µg per ml.

^c From reference (11).

^d Follicles were dissected from ovaries of 1- to 28-day-pregnant rabbits. There was no significant variation in the activities obtained at various times of pregnancy.

^e Follicles were dissected from ovaries of rabbits between 1 and 18 days after the induction of pseudopregnancy with hCG (100 IU per 3-4.5 kg rabbit, iv). These activities did not vary significantly throughout pseudopregnancy.

varying degrees of occupation of the gonadotrophin receptor coupled to AC. By 24 h the AC system in these CL and in those in animals injected with hCG on day 7, had become desensitized, being unresponsive to LH (Fig. 4), and basal AC activity had returned to its pre-injection level (Fig. 4). When ovulation did not ensue (*i.e.*, with single 50 IU sc or ip injection, see *Materials and Methods*), the LH-stimulated AC activity was unchanged by 24 h although basal activity had increased as with ovulatory doses (not shown). Under the assay conditions used, AC system was minimally responsive to PGE₁ or FSH throughout PSP (1.2- to 2-fold stimulation), and a desensitiz-

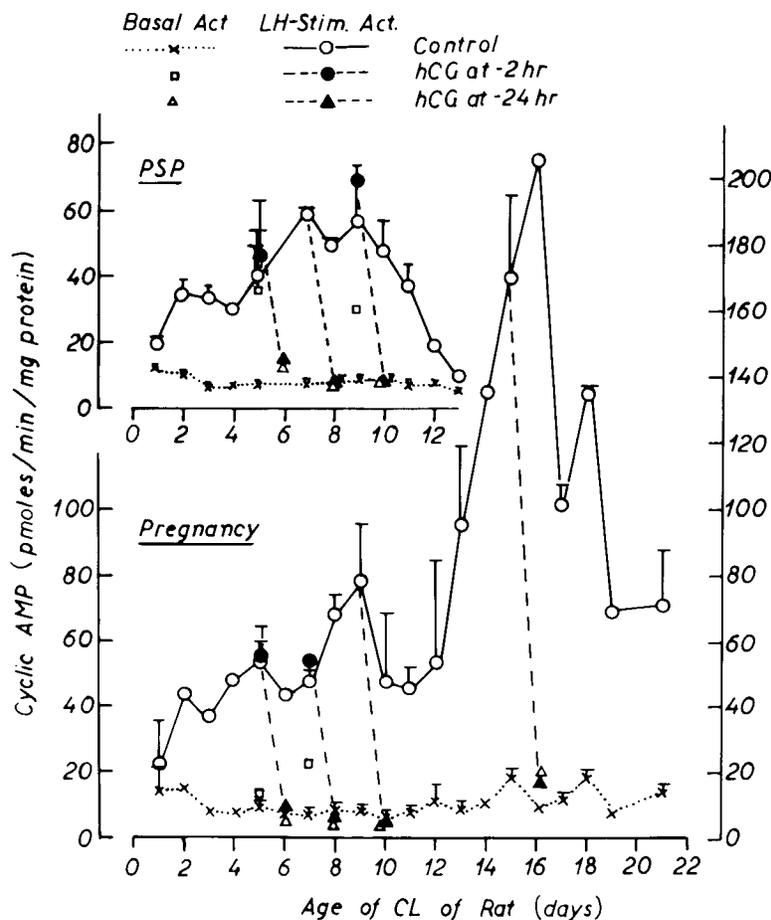


FIG. 4. Effect of hCG (50 IU/rat, ip and sc) injected on various days of PSP (upper panel) and pregnancy (lower panel) on the basal and LH-stimulated adenyl cyclase activity of dissected rat CL. When present, LH was 10 $\mu\text{g/ml}$. For determining the effect of a desensitizing hCG injection, rats were sacrificed 2 or 24 h after hCG injection. Single points represent one assay using CL obtained from 2 rats. Mean \pm SEM is shown where 2 to 4 such assays were performed. For pseudopregnant rats receiving hCG injections 2 h before sacrifice on day 5, and for rats receiving hCG 24 h prior to the assay on days 7 and 9, n equals 2. For pregnant rats receiving hCG injections 2 h before sacrifice on day 5, and for pregnant rats receiving hCG 24 h prior to the assay on days 5 and 7, n equals 2. For all other points, n equals 1. Control data are from reference 12.

ing dose of hCG altered by 24 h neither the responsiveness of the AC system nor the PGE_1 - or FSH-stimulated AC activity.

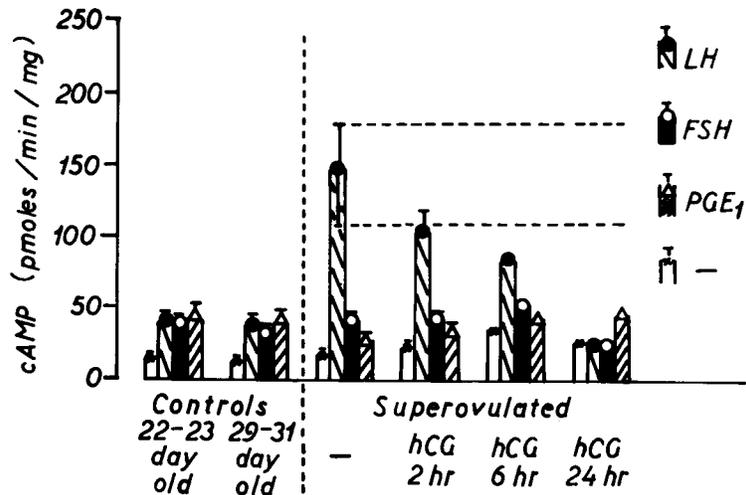
The AC system in ovaries obtained from superovulated rats was not desensitized at 2 h after hCG injection (50 IU, sc), was partially desensitized by 6 h (30% decline in the LH-stimulated AC activity), and was unresponsive to LH and FSH at 24 h (Fig. 5). Basal activities were somewhat increased over preinjection levels. PGE_1 - and FSH-stimulated AC activity were not affected.

The desensitization of the luteal AC in pregnant rats followed a similar time course to that seen in the CL of PSP and of superovulation (Fig. 4, upper vs lower panel), *i.e.*, desensitization could not be detected

at 2 h after hCG injection but was readily seen at 24 h, at which time the AC had become totally desensitized. Basal activity was also increased at 2 h post-hCG injection but not as much as during PSP. Only large doses of hCG which induced ovulation also induced desensitization. This desensitizing effect was not restricted to hCG. Figure 6 shows a dose-dependency curve obtained in 15-day pregnant rats with highly purified hLH (R. J. Ryan batch no. BIB1). It can be seen that, also with this gonadotrophin, large doses (1 to 3 $\mu\text{g}/100$ g of rat) are required to effect desensitization. As in PSP, PGE_1 -stimulated AC activity remained low throughout pregnancy and was not altered by a desensitizing dose of hCG (not shown).

FIG. 5. Adenylyl cyclase activities in rat ovaries obtained from control rats (22 to 23-days-old and 29 to 31-days-old), from superovulated rats (29 to 31-days-old; 50 IU PMSG sc on days 22 or 23 and 50 IU hCG sc on days 24 or 25, respectively), and from superovulated rats which received a "desensitizing" injection of hCG. Activity was determined in the absence (basal) and presence of 10 $\mu\text{g/ml}$ LH, FSH, and PGE_1 . Seven to 9 days following the PMSG injection, the rat ovaries were assayed for adenylyl cyclase activity either without further treatment (-) or having received a second hCG injection (50 IU, sc) 2, 6, and 24 h before sacrifice.

Adenylyl cyclase activities in ovaries of control rats (rats which received no injections), are shown on the left side. Each point represents one assay in which 3 to 6 rats were used. Mean \pm SEM is shown where more than 2 such assays were performed. For 22 to 23 and 29 to 31-day-old control rats, n equals 3. For superovulated rats which received no further injections, n equals 9; for superovulated rats which received hCG 2 h earlier, n equals 6; for superovulated rats which received hCG 24 h earlier, n equals 2. For all other points, n equals 1. Control data are from reference 12.



Discussion

Desensitization of the adenylyl cyclase system in CL has been shown to be induced by the injection of an ovulatory dose of hCG into pseudopregnant and pregnant rats and rabbits, subovulatory doses of hCG being without effect. Similarly, luteolysis can be induced during PSP and pregnancy in both rats and rabbits by the injection of an ovulatory dose of LH or hCG, subovulatory doses of either gonadotrophin being also without effect. Functional luteolysis in the pseudopregnant rabbit is characterized by a decline in progesterone synthesis, demonstrable 16 h, but not 9 h, after an acute injection of LH. Morphological luteolysis follows, characterized by a decline in CL weight, demonstrable 38 h after an LH injection (21). Complete desensitization of the luteal AC system appears to precede morphological luteolysis in pregnant rabbits before day 21 and probably in pseudopregnant rabbits, since in both cases the extent of desensitization 2 h after

an hCG injection indicates a rapid decline in the responsiveness of the AC system. While complete desensitization appears to precede morphological luteolysis, partial desensitization ($\sim 50\%$ reduction of the LH-stimulated AC), as observed in our studies, precedes functional luteolysis. Thus, there would seem to be a close association between hCG-induced luteolysis and hCG-induced desensitization of the AC system in CL. In fact, hCG-induced desensitization of the AC system to LH stimulation in CL may be a marker for the induction of luteolysis by hCG. Not known, however, is whether desensitization of the cyclase system is a prerequisite for hCG-induced luteolysis. To establish such a cause-effect relationship, it will be necessary to show not only that progesterone synthesis declined in a manner parallel to the rate of loss of the AC system, but also, that desensitization of the cyclase system induced by other means leads to luteolysis as well.

Even if hCG-induced desensitization is an initial step in hCG-induced luteolysis, the

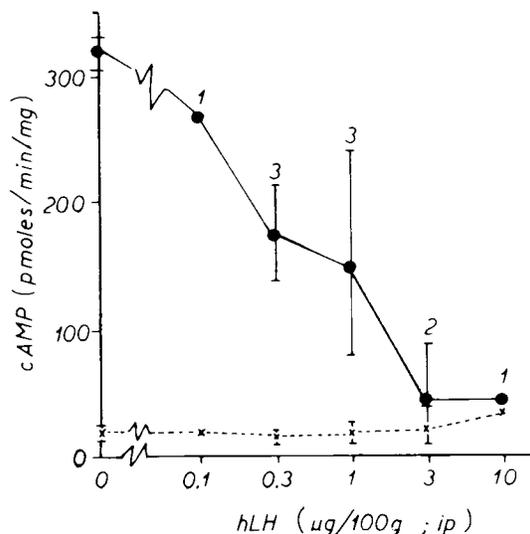


FIG. 6. Effect of the dose of hLH on the responsiveness of rat CL adenylyl cyclase to LH. Activities in homogenates of dissected CL were determined on day 16 of pregnancy, 24 h after the ip injection of hLH (R. J. Ryan batch no. BIB1). Single points represent one assay in which 2 rats were used. Mean \pm range is shown where 2 to 4 such assays were performed. For time 0, n equals 4; for all other points, n is indicated on the figure. Holtzman rats, between 90 and 100 days old by the time of hLH injection, were used for this study. -- \times -- denotes basal activities.

physiological means by which CL normally regress does not appear to be associated with surges of LH and/or desensitization of the AC system. In rabbits, luteal regression at the end of pregnancy or PSP does not seem to be induced by an endogenous surge of LH (22), and in rats, even though parturition is associated with a surge of LH that causes postpartum ovulation (23,24), the loss of progesterone synthesis and, hence, of functional luteolysis, appears to precede this event. On the other hand, ovulatory levels of LH are not always lytic in rats, since an endogenous surge of LH induced on diestrus by the injection of estradiol benzoate on metestrus promotes ovulation (early in proestrus) but does not promote lysis (25). Thus, while desensitization may be closely associated with hCG- and LH-induced luteolysis, it clearly is not the physiologic

means of CL lysis. Whether it is an obligatory response to a surge of LH is not known.

Desensitization of the AC system in the CL of rabbits can be induced by hCG during PSP and pregnancy; however, as shown above, the rate of desensitization seems to vary. In rabbits after day 20 of pregnancy, there is an apparent decrease in the rate of desensitization, measured as an *inability* to detect desensitization at 2 and 24 h after hCG injection, suggesting that some protective effect is conferred on CL in the last 11 days of pregnancy. We found that 3-day-old CL, induced by hCG on day 21 of pregnancy and contained in 24-day pregnant rabbits, were not protected from a second, desensitizing dose of hCG. In fact, the AC system in these CL became desensitized as quickly as estrous follicles, exhibiting at least a 70% decline at 2 h after hCG administration. Furthermore, whatever factor was responsible for delaying desensitization of AC in CL during the last third of pregnancy, it did not seem to affect appreciably the rate of desensitization in follicles contained in the ovaries of pregnant rabbits (Table 2). Perhaps the protection afforded upon CL AC during the last third of pregnancy requires a minimal age of the CL itself, since the pattern of responsiveness of the AC system in CL may be autonomous for the first 6 days, and possibly until implantation, suggested by the lack of a luteolytic effect of estrogen withdrawal on CL steroidogenesis prior to day 7 (26), and by the lack of correlation between progesterone levels and LH-stimulated AC activity prior to implantation (11). Possibly follicles and "young" CL do not possess receptors for the factor which promotes partial protection of the CL from the luteolytic effects of hCG.

In rats, the rate of desensitization of the CL and ovarian AC system appears to be constant throughout pregnancy, PSP, and PMSG-induced "PSP" in prepubertal rats. Compared with the rate of desensitization in rabbit CL, the loss of LH-sensitive cyclase activity in the CL of rats is slower

than in CL both from pseudopregnant rabbits and from rabbits during the first 18 days of pregnancy, but is more rapid than in CL from the last third of pregnancy in rabbits. It might be argued that the delayed rate of desensitization in CL from rats may be due to the less direct injection routes in rats (ip plus SC in rats *vs* iv in rabbits); however, the rate of desensitization of AC is also delayed in proestrous follicles, in which the physiological stimulus for desensitization is delivered iv in the form of an afternoon surge of gonadotrophins (Fig. 2 of the preceding article (12)).

The fate of regressed CL in rats is not known; however, in rabbits, regressed CL are believed to dedifferentiate (27) into non-functional corpora albicantia, and then to be incorporated into interstitial tissue, a structure in rabbits which can literally pour out mg quantities of 20α -hydroxypregn-4-en-3-one in response to LH or hCG stimulation (28,29). We found that the LH-stimulated AC activity in corpora albicantia measured 10 days after the induction of luteolysis was similar to that of interstitial tissue (Fig. 3). We do not know whether the same AC systems are present in interstitial tissue, corpora albicantia, and CL, or whether "new" enzyme systems have developed with the acquisition of function by the interstitial tissue.

In many ways desensitization in the CL resembles that described in previous reports in follicles (11,12). In both tissues only high doses of the stimulating gonadotrophin are effective, the rates vary with the species studied, and the physiological role and implications are not yet clear. We have commented on the fact that hCG-luteolysis appears to be associated with hCG-induced desensitization of AC, that this clearly is not the physiological means of CL regression, and that the role of AC responsiveness during normal regression of CL (at the end of PSP or pregnancy) is not known. Similarly, in follicles, where the end effects of desensitizing doses of hCG and LH are ovulation and luteinization, it is not known

to what extent desensitization is a requirement for proper completion of these effects. Of interest in this regard was the finding that, like desensitization in CL, desensitization in follicles is slower in rats than in rabbits, to the extent that between 8 and 10 h after the endogenous LH surge, *i.e.*, at 2345 h of proestrus, the AC system still exhibited as much as 50% of the LH-stimulated activity it had at noon of proestrus. It seems reasonable to assume, therefore, that *complete* desensitization may not be a prerequisite for ovulation to occur. It remains to be seen whether desensitization is complete *at* ovulation and whether incomplete desensitization would interfere with the proper development of this process. It should be noted that, while desensitization may play an important role in the ovulatory process, it is not always followed by ovulation, as seen in the following 2 examples: (a) large, 2 to 3 mm unovulated (atretic?) follicles dissected from rabbit ovaries 16 h after an hCG injection were found to contain an unresponsive AC system, and (b) unovulated follicles dissected 24 h after an hCG injection in which ovulation was blocked by indomethacin treatment (70 mg indomethacin, SC, with hCG and again 8 h after hCG, 11) were also found to contain an AC system that was desensitized. In CL, desensitization may also not be a sufficient cause for luteolysis to occur, for estrogen administration, known to be luteotrophic and to prevent hCG-induced luteolysis, neither prevented nor reversed hCG-induced desensitization (Table 1). Clearly, further work is needed to establish the physiologic role of desensitization in ovarian tissues.

In summary, throughout these studies (11,12,14,30) we have observed 2 different ways in which AC activity can be modulated: one being stimulation and the other being desensitization. Stimulation of AC by LH or hCG results in production of cAMP (14); is produced with ovulatory and subovulatory doses of gonadotrophin; is demonstrable in disrupted cells as well as in

cell-free membrane preparations (11,12,14, 30); and is responsible for the steroidogenic effects of LH in follicular, luteal, and interstitial tissue, for the luteinizing effects of LH in granulosa cells, and for the initiation of meiosis in oocytes (*cf.* 11). Desensitization of adenyl cyclase by LH or hCG results in the cessation of cAMP production, is not mimicked (in cell-free membrane preparations) by cAMP (30), is induced only with ovulatory doses of hCG or LH (11,12), can be demonstrated *in vitro* in cell-free membrane preparations derived from both pig and rabbit Graafian follicles (30), as well as *in vivo* (11,12), and *may* be a necessary prerequisite for proper luteinization, ovulation, and hCG-induced luteolysis.

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