Mechanism of down regulation of luteinizing hormone receptors and steroidogenesis in corpora lutea

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Abstract. The mechanism of 'down regulation' of luteinizing hormone receptors was investigated in pseudopregnant rats using a modified radioimmunoassay capable of measuring endogenous tissue-bound hormone. Treatment of pseudopregnant animals with a desensitizing dose (desensitization treatment) of human chorionic gonadotropin resulted in a decrease in receptor concentration. This decrease was prevented if the animals were treated prior to the desensitization treatment with indomethacin, an inhibitor of prostaglandin biosynthesis, suggesting a role for prostaglandins in down regulation. The desensitization treatment resulted in a time-dependent decrease in subsequent responsiveness of the tissue to luteinizing hormone. Basal progesterone production rate was also decreased following desensitization. Total tissue cholesterol was found to be decreased following desensitization treatment, without any change in the ratio of free to esterified cholesterol. Mitochondrial cholesterol was significantly reduced and pregnenolone production by the mitochondria of desensitized corpora lutea was also markedly reduced. However, when cholesterol was added to the mitochondria of desensitized corpora lutea, pregnenolone production was increased, reaching values almost equal to that shown by the control mitochondria. These results show that decrease in the responsiveness following desensitization treatment is due to, besides receptor loss, decrease in tissue cholesterol, in particular mitochondrial cholesterol. The cholesterol side chain cleavage activity, although low, appears to be functionally intact; the low activity could be attributed to low levels of mitochondrial cholesterol.

Keywords. Down regulation; desensitization; luteinizing hormone; steroidogenesis.

Introduction

It is a well-recognized property of several hormones and drugs that the primary stimulus modulates the responsiveness of the target tissue to subsequent exposure of the same tissue to the same hormone. This phenomenon is known as 'desensitization' or 'down regulation' and has been demonstrated for several hormones and growth factors. Administration of luteinizing hormone (LH) or human chorionic gonadotropin (hCG) has been shown to cause decrease in LH receptors both in ovaries and testes (Dufau and Catt, 1979). Such a decrease in receptors is also followed by decrease in responsiveness of the tissue to the same stimulus (Dufau and Catt, 1979). It was further demonstrated that steroidogenesis in desensitized tissue could not be stimulated by dibutyryl cAMP or cholera toxin, suggesting a defective steroidogenic pathway (Conti *et al.,* 1977). However, the mechanism by which the hormones cause decrease in the receptors and alter the responsiveness of the tissue is poorly understood. In the present study an attempt has been made to unravel the mechanism by which LH receptor loss and decrease in responsiveness occur using corpora lutea from pseudopregnant rats as the model system.

Abbreviations used: LH, Luteinizing hormone; hCG, human chorionic gonadotropin; oLH, ovine LH; PMSG, pregnant mare serum gonadotropin; RIA, radioimmunoassay; SSC, side chain cleavage.

Materials and methods

Hormones and chemicals

hCG used in this study was a kind gift of Dr C. R. Canfield, USA. Ovine LH (oLH) was a gift of Dr M. R. Sairam, Canada. Pregnant mare serum gonadotropin (PMSG) was purchased from Sigma Chemical Co., St. Louis, Missouri, USA. [^{3}H] Progesterone, [^{3}H]pregnenolone and Na^{125}I were purchased from the Radiochemical Centre, Amersham, UK. All reagents used in this study were of analytical grade.

Animals

Immature female rats 25–26 days of age were rendered pseudopregnant by injecting subcutaneously 15 IU of PMSG followed 56 h later by 50 IU of hCG. Four to five days after hCG treatment the animals were treated with either saline or a desensitizing dose of hCG (referred to hereafter as 'desensitization treatment') and killed after various times depending on the experimental design. Ovaries were removed, and corpora lutea were dissected free of adhering fat using a fine needle and processed according to different experimental designs.

Radioimmunoassay of tissue-bound hCG

Luteal tissue-bound hCG was determined essentially according to the procedure previously used for tissue-bound LH (Muralidhar and Moudgal, 1976a, b) and tissue-bound follicle stimulating hormone (Sheela Rani and Moudgal, 1978). Corpora lutea were homogenized in 0·05 M Tris-HCl, 0·05 M EDTA, pH 7·4, and different aliquots were used for assaying hCG in the tissue by radioimmunoassay (RIA) carried out at 37°C as described by Muralidhar and Moudgal (1976a, b).

Determination of responsiveness of corpora lutea

Responsiveness of the corpora lutea from control and hCG-treated animals to LH was determined by incubating the corpora lutea in Krebs–Ringer bicarbonate medium, pH 7·4, containing 0·05 M Hepes and 0·1 % bovine serum albumin (BSA) in the absence or presence of LH (0·1, 1 and 10 μg/ml oLH) for 2 h at 37°C. Progesterone secreted into the medium was estimated by a specific RIA.

Determination of luteal tissue cholesterol

Corpora lutea were obtained from animals killed 12 or 24 h after desensitization treatment. The corpora lutea from individual animals were suspended in saline and stored frozen until further use. To determine luteal tissue cholesterol and esterified cholesterol, corpora lutea were homogenized in 0·15 M NaCl and total lipids were extracted using a chloroform: methanol mixture (final composition, chloroform: methanol: saline 1:1:0·9). Cholesterol and esterified cholesterol were separated by thin-layer chromatography using hexane: ether: acetic acid (30:10:0·5) as the solvent system and estimated by the method of Glick *et al.* (1964).

Determination of cholesterol side chain cleavage activity

Cholesterol side chain cleavage (SSC) activity of the mitochondria-rich preparation was assayed by estimating pregnenolone produced by the mitochondria. Corpora lutea were homogenized in 0·05 M Tris-HCl, pH 7·4, containing 0·5 mM EDTA and 0·25 M sucrose. The homogenate was spun at 600 g for 10 min. The supernatant was retrieved and centrifuged at 8500 g for 15 min. The pellet was resuspended in the same buffer after washing and used for determining SSC activity. Aliquots of mitochondria (approximately 300 μg protein) were incubated in a total volume of 1 ml of a solution containing 5 mM Ca^{2+}, 5 mM Mg^{2+}, 40 mM nicotinamide, and 10 mM sodium succinate. Cholesterol (100 μM), whenever present, was added in 50 μl absolute ethanol; all other aliquots received the same volume of ethanol. The reaction was initiated by adding the mitochondrial preparation and was continued for 30 min at 37°C. The reaction was terminated by placing the tubes in a boiling water bath for 1 min. Steroids formed were extracted with ether, and separated by thin-layer chromatography using chloroform: ethyl acetate (4:1) as the solvent system. Using this system, it was possible to separate pregnenolone from proges-terone. Pregnenolone was then estimated by RIA using 7-[^{3}H]–pregnenolone as the tracer and a progesterone antiserum with 100% cross-reactivity with pregnenolone.

Results

Demonstration of down regulation of LH receptors

Pseudopregnant rats were treated subcutaneously with saline or a desensitizing dose of hCG (4 μg). The animals were again treated after 48 h with hCG (2 or 4 μg) and killed 3 h later. Tissue-bound hCG was determined by RIA. It was earlier found that maximum uptake of hCG by the ovaries occurs 3 h after a subcutaneous injection of hCG and the uptake was maximal when 4 μg of hCG were administered, suggesting saturation of LH receptors *in vivo*. As shown in table 1, animals that had received desensitization treatment showed a significant decrease in uptake of hCG, demonstrating down regulation of LH receptors following desensitization treatment.

Involvement of prostaglandins in down regulation of LH receptors

The involvement of prostaglandins in the process of down regulation of LH

Table 1. Down regulation of LH receptors.

	hCG (μg) treatment at		Luteal tissue hCG
Group	0 h	48 h	(ng/10 mg tissue)*
A	—	—	0·05 ± 0·11
B	—	2	12·90 ± 2·9
C	—	4	19·40 ± 2·4
D	4	—	0·19 ± 0·03
E	4	2	2·74 ± 1·17
F	4	4	2·47 ± 0·65

*Values are mean ± SD, n = 4.
Group E significantly different from Group B, $P < 0.001$.
Group F significantly different from Group C, $P < 0.001$.

receptors was investigated by studying the effect of a prostaglandin synthesis inhibitor on hCG-induced receptor loss. The animals were administered 1 and 4 μg of hCG as a desensitization treatment and uptake of hCG 3 h following the administration of 5 μg of hCG 48 h after desensitization was determined. One group of animals was treated with 500 μg indomethacin 1 h prior to desensitization treatment and hCG uptake by luteal tissue was determined as described above. As shown in table 2, the desensitizaton treatment resulted in a dose-dependent decrease in hCG

Table 2.   Effect of indomethacin on hCG-induced receptor loss.

| | Treatment at | | | Luteal tissue hCG (ng/10 mg tissue)* |
| | −1 h | 0 h | 48 h | |
Group	Indometha-cin (μg)	hCG (μg)	hCG (μg)	
A	—	—	5	$9 \cdot 5 \pm 1 \cdot 5$
B	—	4	—	$0 \cdot 45 \pm 0 \cdot 05$
C	—	4	5	$2 \cdot 15 \pm 0 \cdot 18$
D	500	4	—	$0 \cdot 38 \pm 0 \cdot 05$
E	500	4	5	$2 \cdot 9 \pm 0 \cdot 13$
F	—	1	—	ND
G	—	1	5	$3 \cdot 5 \pm 0 \cdot 3$
H	500	1	5	$8 \cdot 6 \pm 1 \cdot 45$

*Values are mean ± SD, n = 4.
Significance of difference: C *vs* A, $P < 0 \cdot 001$; G *vs* A, $P < 0 \cdot 001$; E *vs* C. $P < 0 \cdot 005$; H *vs* G, $P < 0 \cdot 005$.
ND, Not determined.

uptake by the corpora lutea, suggesting loss of LH receptors. Indomethacin treatment reduced receptor loss. This effect was only marginal when the desensitizing dose was 4 μg but completely prevented receptor loss when the desensitizing dose was 1 μg.

Effect of desensitization treatment on responsiveness of the corpora lutea

Corpora lutea obtained from rats after various times following desensitization treatment were incubated with different concentrations of oLH or without oLH for 2 h at 37°C. Progesterone secreted into the medium was estimated by RIA. As shown in figure 1, the control corpora lutea (0 h) responded to LH *in vitro* by producing increased progesterone. However, the corpora lutea obtained 4, 12, 24 and 48 h after the desensitizing dose did not show any responsiveness to LH added *in vitro*. The corpora lutea obtained 4 h after desensitizing treatment showed increased basal production *in vitro* but did not show any further responsiveness. Corpora lutea obtained 12, 24 and even 48 h after the desensitization treatment showed decreased basal production of progesterone.

Effect of desensitization treatment on sterol levels of luteal tissue

Endogenous cholesterol and esterified cholesterol levels were determined in luteal tissue 12 and 24 h following desensitization treatment. Untreated animals served as
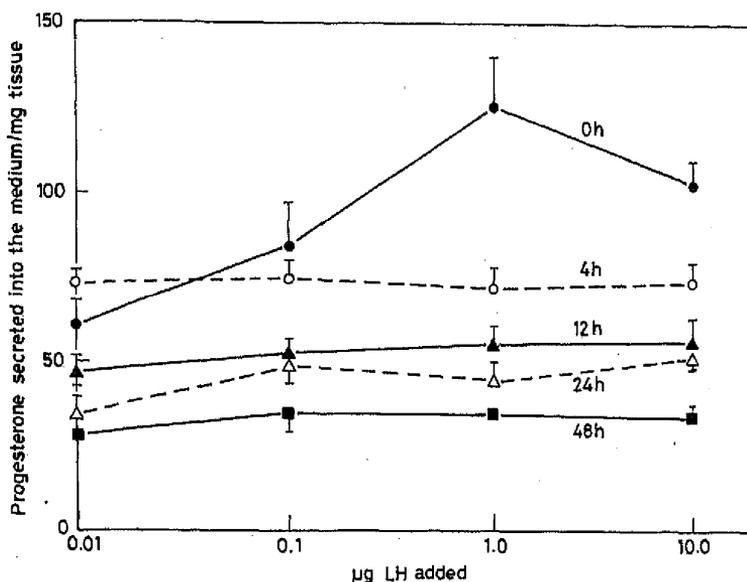
Figure 1. *In vitro* responsiveness of the corpora lutea following a desensitization treatment. Corpora lutea were obtained from animals 4, 12, 24 and 48 h after treatment; 0 h indicates untreated animals (control). Each point is the mean ± SD of triplicates.

controls. As shown in table 3 there was a significant decrease in cholesterol levels ($P < 0.05$), although by 24 h the cholesterol levels appear to be higher than at 12 h

Table 3. Endogenous free and esterified cholesterol in control and desensitized corpora lutea.

Time after hCG treatment (h)	Cholesterol (μg/mg tissue)	Esterified cholesterol as cholesterol equivalent (μg/mg tissue)	Total cholesterol* (μg/mg tissue)
0	0·99 ± 0·13	2·36 ± 0·24	3·37 ± 0·37
12	0·63 ± 0·12	1·54 ± 0·36	2·46 ± 0·40
24	0·73 ± 0·14	1·88 ± 0·29	2·61 ± 0·40

*Values are mean ± SD, n = 4.
Significance of difference: Cholesterol 12 h *vs* 0 h, $P < 0.05$; cholesterol 24 h *vs* 0 h, not significant; cholesterol ester, 12 h *vs* 0 h, $P < 0.05$; cholesterol ester 24 h *vs* 0 h, not significant; total cholesterol 12 h *vs* 0 h, $P \simeq 0.05$.

and not significantly different from 0 h levels. A similar pattern was observed for esterified cholesterol, and for free and esterified cholesterol taken together (total cholesterol). However, total cholesterol present in the mitochondria obtained from pooled corpora lutea was markedly reduced even 24 h after the desensitization treatment (table 4).

Effect of desensitization treatment on cholesterol side chain cleavage activity

Mitochondria prepared from corpora lutea obtained from control and hCG-desensitized animals were used for determining cholesterol side chain cleavage

Table 4. Cholesterol levels in mitochondria-rich preparation obtained from control and desensitized corpora lutea.

Treatment	Mitochondrial cholesterol (µg/mg protein)
Saline	27·6
hCG (4 µg)	13·95

Animals were killed 24 h after treatment.

activity. As shown in table 5, pregnenolone production by mitochondria from hCG-treated rats was significantly lower than that by control mitochondria. While addition of exogenous cholesterol to mitochondria from control corpora lutea did

Table 5. Cholesterol side chain cleavage activity in control and desensitized luteal mitochondria.

Group	Pregnenolone formed (pmol/mg protein)*
A Control luteal mitochondria	205·9 ± 37·4
B Desensitized luteal mitochondria	67·4 ± 20·4
C Control luteal mitochondria + cholesterol	180·1 ± 40·0
D Desensitized luteal mitochondria + cholesterol	200·1 ± 51·6

Animals were killed 24 h after desensitization treatment or saline injection (control).
*Values are mean ± SD, n = 4.
Significance of difference: B *vs* A, $P < 0.001$; D *vs* B, $P < 0.025$; C *vs* D, not significant.

not enhance pregnenolone formation, the mitochondria from desensitized luteal tissue showed stimulated production of pregnenolone and this was equal to that produced by the control mitochondria.

Discussion

In the studies presented above an attempt has been made to understand the mechanism of down regulation of LH receptors and the subsequent reduction in responsiveness of the corpora lutea. The down regulation of receptors was demonstrated using an RIA system extensively validated earlier (Muralidhar and Moudgal, 1976a, b; Sheela Rani and Moudgal, 1978; Dighe, 1982) for measurement of physiologically active LH bound to receptors. With this assay system it has been demonstrated here that decrease in hormone uptake following desensitization treatment was indeed due to a true loss of the receptors and not due to decrease in binding of the hormone to the receptors caused by higher occupancy of the receptors after the desensitization treatment.

Prostaglandins have been implicated in bringing about luteolysis in several species Labhsetwar, 1975; Zor and Lamprecht, 1977; Behrman, 1979). Prostaglandins have also been shown to cause reduction in LH receptor concentration (Hichens *et al.,* 1974; Grinwich *et al.,* 1976a, b; Behrman and Hichens, 1976; Behrman *et al.,* 1978). In the present study it has been shown that inhibition of prostaglandin biosynthesis by indomethacin treatment prevented hCG-induced receptor loss. The protection provided by indomethacin was dependent on the dose of indomethacin, 500 μg being the most effective dose (data not shown). At this dose inhibition of receptor loss was partial if the desensitizing dose of hCG was 4 μg but complete inhibition of receptor loss was observed when the desensitization dose of hCG was reduced to 1 μg. These results provide confirmatory evidence for the involvement of prostaglandins in regulating luteal cell LH receptor concentration. One of the events that occur following binding of the hormone to the receptor is perhaps stimulation of prosta-glandin biosynthesis which in turn initiates down regulation of receptor. Haour *et al.* (1979a, b) have noted that in the Leydig cell prostaglandin levels increase 6 h following hCG administration. In the present study it was also found that administration of indomethacin 90 min or 6 h after desensitization treatment did not prevent the receptor loss (data not shown). Thus receptor loss, once initiated, could not be prevented by indomethacin administration.

A decrease in the receptor concentration was also accompanied by decrease in responsiveness of the corpora lutea. Progesterone production decreased in a time-dependent fashion and was reflected both in basal rate of progesterone production as well as in response to LH *in vitro*. This suggested that in addition to receptor loss significant alterations in the progesterone biosynthetic pathway should be occurring. There is a large body of evidence to show that loss of LH receptors is also accompanied by several lesions in the steroid biosynthetic pathway. Luteal adenylyl cyclase activity was found to be decreased following administration of a desensitizing dose of hCG to pseudopregnant rabbits (Hunzicker-Dunn and Birnbaumer, 1976). Further, it was shown that desensitized Leydig cells responded to cholera toxin with increased cAMP production, but failed to respond in terms of testosterone production (Tsuruhara *et al.,* 1977). It has also been observed that desensitized luteal cells do not respond to cholera toxin and dibutyryl cAMP in terms of progesterone production (Conti *et al.,* 1977). In the case of Leydig cells the lesion in testosterone biosynthesis was identified as a decrease in the activities of the enzymes 17–20 lyase (Cigorraga *et al.,* 1978) and 17α hydroxylase (Chaslow *et al.,* 1979). However, such defects have not been identified in luteal cells. The levels of cholesterol and esterified cholesterol were found to be decreased in corpora lutea obtained from hCG-treated animals. However it must be noted that there was no decrease in the ratio of free to esterified cholesterol. Down regulation, as described here, results in decrease in progesterone biosynthesis and this is similar to that occurring in corpora lutea undergoing functional luteolysis. Blockade or reduction in endogenous LH availability brought about by LH antibody treatment resulted in significant accumulation of esterified cholesterol in the luteal tissue (Moudgal *et al.,* 1972; Behrman *et al.,* 1972; Mukku and Moudgal, 1980) and this was unlike that observed in desensitized corpora lutea. Corpora lutea undergoing luteolysis remained responsive to added LH *in vitro* in terms of progesterone production, though this declined with time (Mukku and Moudgal, 1980); however, the desensitized corpora lutea did not respond to LH at all. These results suggest that there is a fundamental

difference between luteolysis brought about by LH removal and desensitization brought about by LH receptor down regulation.

The desensitized corpora lutea also showed marked decrease in mitochondrial cholesterol. Only part of the mitochondrial cholesterol is available to the side chain cleavage activity (see Dorrington, 1977). Hence the effect of a decrease in the cholesterol level of mitochondria on side chain cleavage activity is probably enhanced further. There was a significant decrease in pregnenolone production by mitochondria from desensitized corpora lutea. This defect in pregnenolone biosynthesis could be ameliorated by addition of cholesterol to the mitochondrial preparation: pregnenolone production by mitochondria from desensitized corpora lutea in the presence of exogenous cholesterol was nearly equal to that by control mitochondria. These results suggest that the cholesterol side chain cleavage activity is probably functionally intact, and the decrease in progesterone biosynthesis could be due to the decrease in the cholesterol pool in mitochondria which acts as the substrate for cholesterol side chain cleavage enzymes. Replenishment of the mitochondrial cholesterol pool may take a much longer time than that of tissue cholesterol. Levels of tissue cholesterol had returned to nearly control levels 24 h following desensitization treatment, but cholesterol in the mitochondria remained markedly reduced. There was no change in 3β-hydroxy-steroid dehydrogenase in desensitized corpora lutea but a significant increase in cAMP phosphodiesterase activity was noted (data not shown). This defect can cause further decrease in progesterone biosynthesis.

Thus down regulation of luteal tissue following administration of hCG occurs at at least two different levels—(i) loss of receptors which is perhaps mediated by prostaglandins and (ii) decrease in the cholesterol pool resulting in decrease in progesterone production.

Acknowledgements

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