An immunological approach to study gonadotropin action at the cellular and molecular level

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Introduction

The last two decades have seen a rapid development of a variety of new techniques and probes to understand the regulatory functions of hormones. One such tool is antibodies against hormones. Antibodies against the gonadotropins Lutropin (LH) and Follitropin (FSH), in addition to showing hormonal specificity, react with LH and FSH of a variety of other species (1–3). This property has been found extremely useful in developing heterologous radioimmunoassay (RIA) systems (4) and also for neutralizing endogenous LH or FSH at critical periods of reproduction in a variety of animals (5, 6).

The classical approach to study the action of gonadotropins has been to use the hypophysectomized animal model and administer as required hormones having LH or FSH activity. Such an approach would result in the removal, at a stroke, of all the pituitary hormones other than the supplemented gonadotropin. In this procedure the permissive or antagonistic effect that the other pituitary hormones may have on the gonadotropin action when animals are in a normal physiological state is mostly overlooked. In contrast, use of an antibody, whose specificity can readily be manipulated, ensures that a physiologically normal animal can be deprived of a single hormone at any given time for a fixed period. This approach has been taken advantage of in elucidating, beyond reasonable doubt, the luteotropic activity of LH in the cycling and pregnant rat, hamster, monkey and the rabbit (7, 8). Use of a specific FSH antibody similarly helped to establish, for the first time, the need for this hormone in maintaining spermatogenesis and fertility in the primate (9–12). Further, specific gonadotropin antibodies have also been employed successfully in studying events like follicular maturation, ovulation, implantation and also as a vaccine to prevent or terminate pregnancy. This aspect has been reviewed in depth earlier (7,13,14). In the present paper emphasis will be laid on discussing in some depth how specific LH and FSH antibodies have been used to further our knowledge of the mechanism of action of these hormones at the cellular level.

Characterization of Antibodies

The use of gonadotropin antibodies as specific hormone antagonists necessitated the development of methods to characterize these antibodies for

specificity. Even though the LH or FSH used as immunogen in earlier years was relatively pure, it was not possible to obtain antisera free of contaminating antibodies against the other pituitary proteins. This was particularly true of FSH antisera, which used to be contaminated to a large extent with
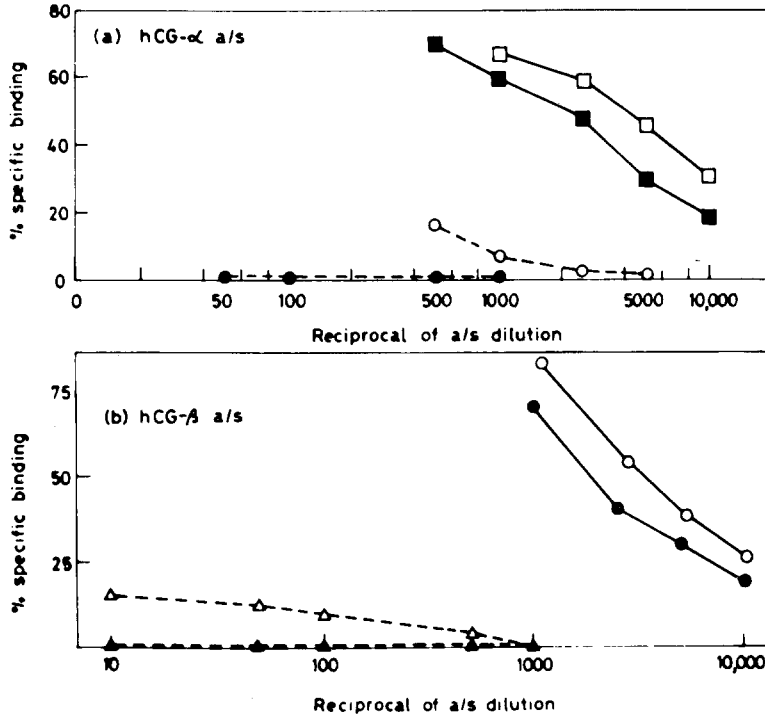

Fig. 1. Characterization of hCG-*alpha* and -*beta* antiserum (a/s). Different dilutions of crude and processed (passed twice through Sepharose to which the counter subunit is attached). hCG-*alpha* or -*beta* antisera (100 μl of each dilution) were incubated with ^{125}I-hCG, ^{125}I-hCG-*alpha*, or ^{125}I-hCG-*beta* for 12 h at 37°C. The antigen–antibody complex was precipitated by adding 100 μl each of 1/50 diluted NRS and goat antibody to rabbit gamma globulin and continuing incubation for 12 more hours. Binding was determined as described in (19). Results have been expressed as bound c.p.m./total c.p.m. × 100 versus reciprocal of antiserum dilution.

(a) hCG-*alpha* antisera.
    □, ^{125}I-hCG + crude antiserum.
    ■, ^{125}I-hCG + processed antiserum.
    ○, ^{125}I-hCG-*beta* + crude antiserum.
    ●, ^{125}I-hCG-*beta* + processed antiserum.

(b) hCG-*beta* antisera.
    ○, ^{125}I-hCG + crude antiserum.
    ●, ^{125}I-hCG + processed antiserum.
    △, ^{125}I-hCG-*alpha* + crude antiserum.
    ▲, ^{125}I-hCG-*alpha* + processed antiserum.

(After Dighe & Moudgal [19]).

antibodies to LH and other non-specific proteins. The contaminating anti-bodies had to be removed by manual addition of the contaminants in repeated small quantities (6). With the advent of immunoaffinity procedures, this is totally obviated (15). Such procedures have been used to cleanse FSH antiserum of LH antibody contamination (10), and FSH, before being used as an immunogen, of LH contamination by passing FSH through an LH antibody coupled CNBr or epoxyactivated Sepharose column (16).

The cross-reactivity of FSH antibodies with LH can also be ascribed to sharing of common *alpha* subunit between LH, FSH and TSH of any given species. However, because the *alpha*-subunit is a poor immunogen, unless deliberate attempts are made to produce antibodies to it, over 90% of the antibodies produced to intact undissociated hormone are directed towards the *beta* subunit [for example, oLH (17, 18)]; that is, the antibodies are conformation-specific. Antisera raised to individual *alpha* and *beta* subunits of human Chorionic Gonadotropin (hCG) have been made specific by treating the individual antisera with the counter subunit coupled to immuno-affinity matrix (19). Such characterized antisera still react with the native undissociated hormone (Fig. 1). Attempts have been made to predict, on the basis of hydrophilicity, the number of potential antigenic determinants hCG or hLH may possess (20). Monoclonal antibodies to hCG have also been produced (21), and the use of these in mapping the receptor binding domain(s) of the hormone will be commented upon later. FSH provides mostly confor-mation-specific antibodies (22), the cross-reactivity with the free *beta* subunit being minimal (23). Further, immunization with FSH, unlike LH or hCG, results in antibodies which form only a soluble complex (24).

The gonadotropin antisera thus far used for neutralization of hormonal activity have been polyclonal in nature and have been produced in a variety of animals which include rabbits, monkeys and horses. While most gonado-tropic antisera are capable of neutralizing the hormonal activity, some investigators have observed an accentuation of the hormonal response with the addition of antibodies (25). Due to incomplete information on the characterization of such antibodies it is difficult to pinpoint the reasons for such observation.

Use of Antisera in Studying the Dependency of Gonadal Steroidogenesis on LH and/or FSH

The gonads of both the male and the female of a variety of species (e.g. rat, mouse, hamster, rabbit and monkey) are very sensitive to deprivation of LH support. For instance, it has been shown in the case of the female pregnant hamster (Fig. 2) and the cycling monkey (Fig. 3) that within a few hours of antiserum injection, the steroid output from the luteal compart-ment of the ovary drops significantly: the effect of this is termination of either pregnancy or cycle (29, 54). The resilience of the luteal tissue has been shown by using an antiserum of low titer. In such a case the reduction in progestin output is transient, the luteal tissue returning to normalcy once the antibody activity is spent (26). For the luteal tissue of the monkey to become totally insensitive to LH action, it is necessary to deprive the animal of LH support for at least 48 h. LH has also been shown to stimulate choles-
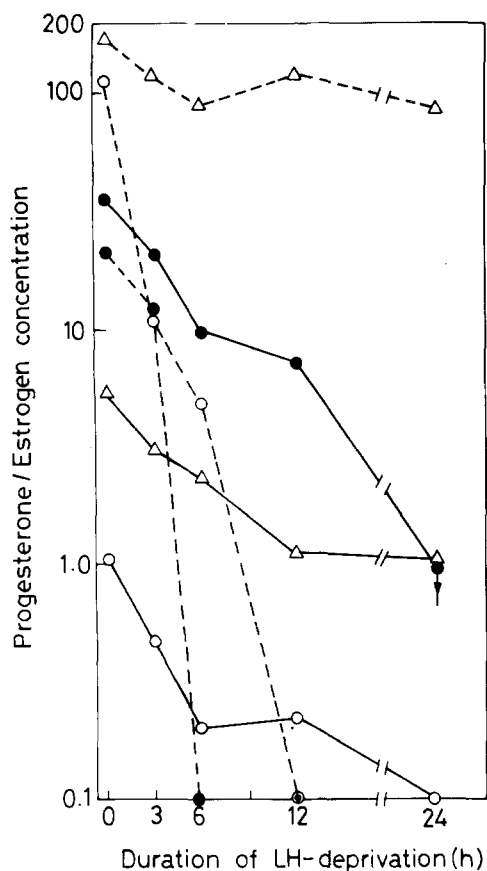
Fig. 2. Composite diagram representing the effect of LH antiserum on progesterone (P) and estrogen (E) levels in serum (△, ng/ml), in corpus luteum (●) and in the non-luteal compartment (○) of the ovary of the pregnant hamster. Solid lines represent P (ng/mg) while broken lines represent E (pg/mg) levels. (After Mukku & Moudgal [29]).

terol ester hydrolysis (27). In keeping with this is the observation that following LH deprivation the cholesterol ester levels of the luteal tissue rise rapidly (28,29), this effect being much more marked than is altered progesterone production. Interestingly, even when the luteal tissue is seemingly insensitive to LH deprivation in terms of progesterone synthesis, its basic dependence on LH is demonstrable if cholesterol ester levels are monitored (52). This is best illustrated by studying the effect that LH deprivation produces in the luteal tissue of rats on different days of pregnancy (Table 1). In contrast to these observations, the rat corpora lutea desensitized by treatment with a high dose of hCG show some decrease in the total cholesterol level without any change in the free/esterified cholesterol ratio (Table 2). The progesterone production in desensitized corpora lutea could be stimulated by the addition of cholesterol (30), while the luteolytic corpora lutea are unable to do so in spite of higher cholesterol levels. It appears from these studies that the biochemical events responsible for inhibition of steroidogenesis of corpora lutea deprived of LH are different from that of corpora lutea made deficient in the LH receptors. Within 1 h of injection of LH antibodies into the male rodent or monkey, testosterone production is drastically reduced, thus showing that even the male gonadal steroidogenesis is dependent upon continuous LH support (31). The specificity of the anti-
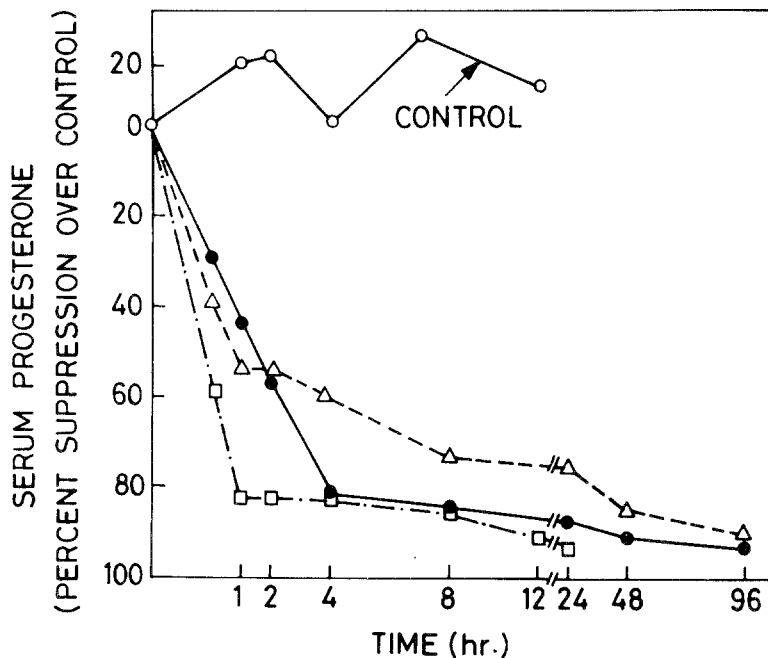
Fig. 3. Effect of injection of a chimpanzee antiserum to oLH *beta* subunit on serum progesterone levels of cycling bonnet monkeys. 5 ml of antiserum was injected i.v. on day 18 of cycle, and blood samples collected at times thereafter were analysed for progesterone. Controls received an equivalent volume of normal chimpanzee serum. n of controls = 2; exptl = 3. (After Moudgal et al. [54]). The other three curves represent decrease in serum progesterone after antiserum treatment of three individual monkeys, nos. 80 (△), 48 (●) and 508 (□).

Table 1.   Effect of LH deprivation on the corpus luteum levels of progesterone and cholesterol on different days of pregnancy in the rat

0.3 ml of LH antiserum was injected into experimental animals, and the animals were autopsied 24 h later. Controls received an equal volume of normal rabbit serum. The free and esterified sterol levels were measured by appropriate methods described elsewhere. Results based on Mukku and Moudgal (52). n of each group = 4; values are means ± S.D.

Treatment on days of pregnancy	Progesterone (ng/mg)		Free cholesterol (μg/mg)		Esterified cholesterol (μg/mg)	
	Control	Antiserum	Control	Antiserum	Control	Antiserum
6	83.7 ± 14.3	102.2 ± 10.0	1.2 ± 0.0	2.2 ± 0.3	1.3 ± 0.4	17.5 ± 3.4
7	110.8 ± 22.6	32.6 ± 1.9	1.5 ± 0.0	2.2 ± 0.2	1.6 ± 0.3	15.6 ± 1.8
8	79.3 ± 7.6	6.3 ± 1.1	1.2 ± 0.1	2.5 ± 0.3	1.4 ± 0.1	13.4 ± 1.9

body effect is apparent from the fact that neither normal serum nor FSH antiserum had an effect on the LH-specific luteal or Leydig cell function.

Antibodies to FSH have been used in studying the specific role of FSH in (a) ovarian granulosa cell multiplication (32, 56), (b) regulating the aromatiz-

Table 2.   Endogenous free and esterified cholesterol in control and desensitized corpora lutea

Immature female rats made pseudopregnant by sequential treatment with PMSG and an ovulatory dose of hCG were treated with a desensitizing dose (4 μg) of hCG 4 days following the ovulatory trigger. Rats were killed at 0, 12 and 24 h after hCG injection, corpora lutea isolated from individual rats and free and esterified cholesterol measured according to the procedure outlined earlier (29). Values are means ± S.D. of 4 animals per group.

h after hCG treatment	Cholesterol (μg/mg tissue)	Esterified cholesterol cholesterol equivalent (μg/mg tissue)	Cholesterol* total (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 ± 0.45
24	0.73 ± 0.14	1.88 ± 0.29	2.61 ± 0.40

* Value obtained by adding free cholesterol and esterified cholesterol.

ing ability of the granulosa cells (53) and (c) regulation of LH receptors (33). In vitro studies with the non-luteal portion of ovaries from the FSH antiserum treated hamster with and without FSH have shown that aromatization of androgens to estrogen is an FSH-dependent phenomenon (53). The dependence of the follicular apparatus on FSH seems to vary depending on the species and can be determined with FSH antiserum. While in the cycling hamster follicular maturation is dependent upon FSH up to diestrous evening (32), in the monkey the growing follicle appears to become independent after day 8 of the cycle (34). Interestingly in the rabbit, which is an induced ovulator, the ovulatory action of LH can be blocked by giving FSH antiserum 6 h prior to the ovulatory trigger (35). Follicular atresia, a lytic process signalling termination of follicular development of a majority of follicles, can be brought about by injection of FSH antiserum. In the rat it has been observed that a lack of FSH leads to inhibition of estrogen production and this in turn brings about an increase in lysosomal enzymes responsible for cell lysis (36).

Use of Antibodies in Studying Gonadotropin–Receptor Interaction

Currently the binding of ^{125}I labelled gonadotropin to the membrane component of the cell is used as a measure of receptor binding. The first demonstration that physiologically active LH can bind to receptors was that of Moudgal et al. (37), using a mouse Leydig tumor cell system and a modified RIA capable of measuring tissue bound hormone. They showed that the Leydig cells bind LH in a dose-dependent manner and that this can be correlated to testosterone production. A similar observation has been made with respect to FSH binding to rat ovarian granulosa cells and estrogen production (44). A direct correlation between FSH bound to the receptor and estrogen produced is evident from Fig. 4. The modification in the RIA essentially consisted of incubating tissue homogenates at 37° instead of 4°. Incubation at 37° for 12 h tends to inactivate the receptor, with the result that the
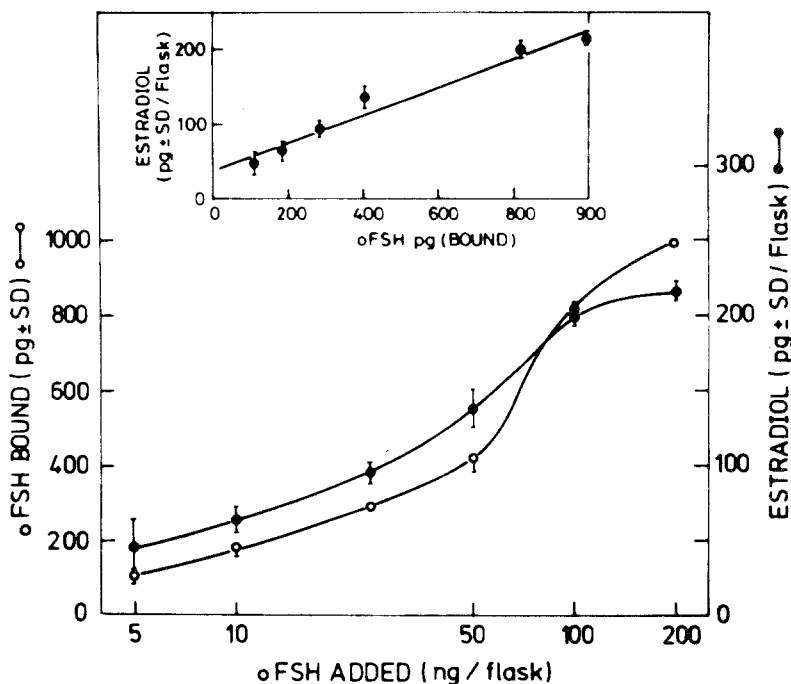
Fig. 4. Establishment of correlation between binding of FSH to rat granulosa cells and accompanying response. FSH and estradiol were measured using appropriate RIA. Inset provides a plot of the FSH bound to the cells against the estradiol produced. Regression analysis of the data gives a slope of 0.19 and an intercept of 36.39; the correlation coefficient = 0.96. (After Vidyashankar & Moudgal [44]).

hormone released from receptor binding is now available for binding to the antibody. This RIA system was subjected to extensive testing and validation (38,39) and was used to show that the *beta* subunit of LH is capable of binding to the cell and that this binding inhibits subsequent binding and response to intact LH (40). Essentially similar observations have been made by Dighe et al. (41) using hCG and its *beta* subunit and ovarian minces. The ability of the hCG *beta* subunit or its fragment to bind to the cellular receptor and evoke a response has been confirmed by others (42, 43). The modified RIA system has also been employed to determine the actual amount of FSH bound to various compartments of the ovary during the estrous cycle in the golden hamster (55). This study demonstrated that serum levels of FSH need not necessarily reflect the levels present in the tissue, the latter being a function of the FSH receptor concentration in the ovary at a given time (Fig. 5). The high levels of FSH seen in the ovary on the evening of diestrous-2 appears to be a reflection of receptor concentration and this occurs despite serum FSH being very low (Fig. 5). This assay has also been used to quantitate the FSH receptor concentration in the granulosa cells of immature estrogen primed rats (44). Vidyashankar and Moudgal (44) reported a value of 375 FSH binding sites/cell and a Kd of 3.03×10^{-10} M, and these are similar to that obtained using ^{125}I FSH (45,46). The modified RIA system has also
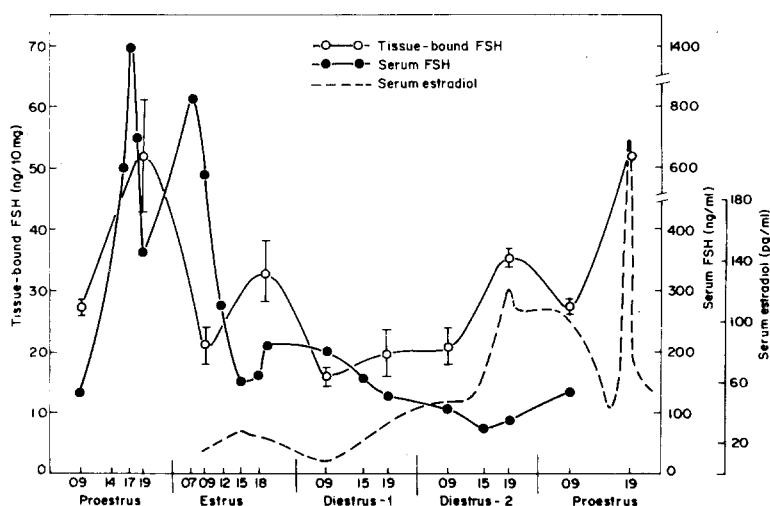
Fig. 5. Pattern of ovarian and serum FSH levels throughout the estrous cycle of the hamster. FSH in the non-luteal ovarian tissue and in serum was determined by RIA carried out at 37°C. The values are expressed in terms of NIAMDD rat FSH/RP standards. The dashed line represents the serum estradiol levels in the cycle hamster. (After Sheela Rani & Moudgal [55]).

Table 3.   Demonstration of 'down regulation' of LH receptors using the RIA method

Immature female rats made pseudopregnant by sequential treatment with PMSG and an ovulatory dose of hCG were treated with a desensitizing dose (4 μg) of hCG 4 d following the ovulatory trigger (t = 0 h). Control animals received saline. 48 h later the animals were treated with graded dose of hCG and killed at the 51st h, luteal tissue separated and hCG bound to it measured by the modified RIA. n of each group = 4.

hCG (μg) treatment at		Luteal tissue hCG (ng/10 mg tissue; mean ± S.D.)
0 h	48 h	
—	—	0.05 ± 0.11
—	2	12.90 ± 2.9
—	4	19.40 ± 2.4
4	—	0.19 ± 0.03
4	1	1.72 ± 0.66
4	2	2.74 ± 1.17
4	4	2.47 ± 0.6

been used to demonstrate 'down regulation' of LH receptors (Table 3) as well as reinduction of receptors (30). Interestingly this study also showed that the down regulation of LH receptors is probably a prostaglandin mediated event since administration of indomethacin prior to administration of desensitizing doses of hCG prevented the decrease in the LH receptors. Even though the RIA method appears laborious compared to the simplistic use of ^{125}I-labelled hormones, it provides each time a quantitation of physio-

logically active hormone bound to the receptor. Verification of basic concepts by this method is desirable as we are not sure if radioiodination, like any other chemical modification, alters the physiological state of the hormone.

The response that follows binding of a gonadotropin to its receptor in most cases can be terminated by the addition of specific antisera in minute quantities to the incubation mixture. Thus in the case of the mouse Leydig cell, the cAMP production in response to LH could be blocked by addition of LH antiserum 15 min following exposure of cells to LH (47). In the case of FSH, using rat granulosa cells it has been shown that addition of FSH antiserum any time during a 4 h incubation period stops further synthesis of estrogen (44). Interestingly the ability of PMSG to induce FSH and LH receptors in granulosa cells of the female rat is also dependent upon the continuous presence of the hormonal stimulus (33), antiserum injection resulting in cessation of the inductive process. Using this model system it has been possible to calculate the half-life of the induced receptors, and this ranges between 7 and 8 h (33).

In addition to affinity-labelling techniques, the common approach used in studying the structure–function relationship of hormones is by modifying the hormone chemically, and ascertaining how biological properties of the derivative have been altered. An alternative probe of considerable advantage in studying hormone receptor interaction is the use of specific antibody. Hormone receptors as well as hormone antibodies exhibit specific binding sites to the gonadotropin under test, but these binding sites need not necessarily be identical. Both polyclonal and monoclonal antibodies to the individual subunits have been used to obtain an insight into the hormonal domain(s) involved in receptor interaction. Our studies with the *beta* subunit of LH/hCG had revealed that this subunit has the minimal topography necessary for it to be recognized by the receptor. So it was envisaged that the first major interaction between the receptor and the hormone could perhaps be taking place largely through the *beta* subunit. If this were so, a large portion of the *beta* subunit of the native hormone would be expected to be buried inside the receptor socket while the *alpha* subunit would remain outside. The correctness of this hypothesis was verified by determining which of the two subunit antisera could inhibit the response to receptor bound hormone. The characterization of antisera raised to individual subunits for specificity was as outlined in an earlier section. Based on the ability of intact hCG and its subunits to displace ^{125}I hCG from binding to the subunit-specific antisera, it was concluded that these antisera bound hCG through the respective subunit portion of the intact hormone molecule. The antisera used in the present study were equipotent with respect to their ability to bind hCG (Fig. 1). When the effect of these antibodies on hCG stimulated testosterone production was studied, it was found that the hCG *alpha* antiserum was significantly more effective in blocking the response to hCG than hCG *beta* antiserum (Fig. 6). This effectiveness of the antiserum in blocking the response diminished as the time of association of the hormone with the cellular receptor was prolonged, suggesting that antigenic determinants of receptor-bound hormone available for antibody binding diminish with time. The observation that hCG *beta* antiserum, even at the earliest time point, was markedly less
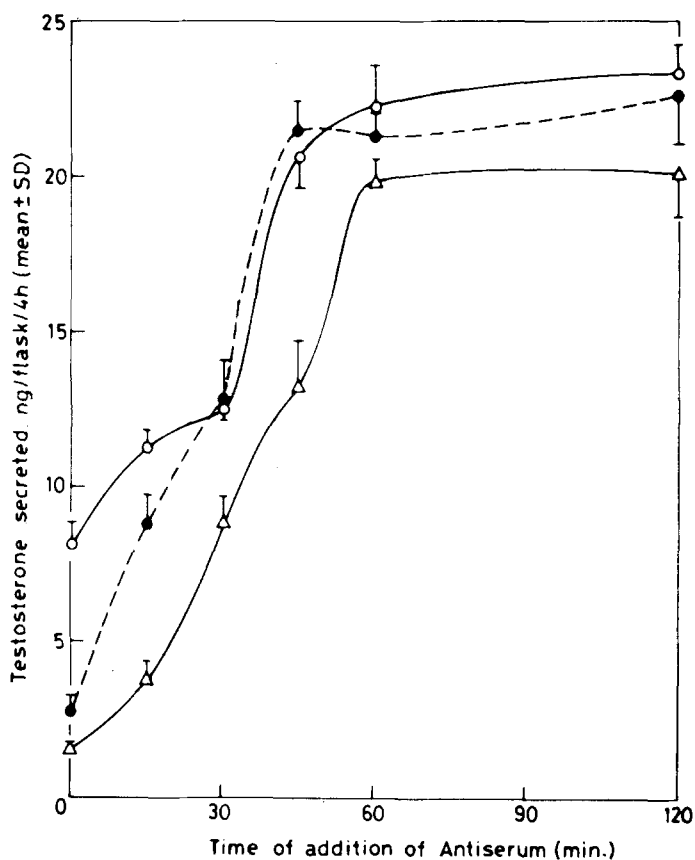
Fig. 6.   Effect of addition of antisera on response to hCG as a function
of the time of addition of antisera. Leydig cells were incubated with
1000 pg hCG. 100 μl of 1:10 dilution of each antiserum was added to
the flasks at various time points. Incubation was carried out at $34°C$
for 4 h. Testosterone secreted into the medium was estimated by RIA.
Values are means ± S.D. of triplicate flasks. Testosterone produced
when no antiserum was added was 24.27 + 3.10 ng/flask/4 h. △, hCG
antiserum; ●, hCG-*alpha* antiserum; ○, hCG-*beta* antiserum. (After
Dighe & Moudgal [19]).

effective in blocking response compared to hCG *alpha* antiserum suggests
that (a) the initial recognition of hCG by its receptor is achieved via binding
sites located in the *beta* subunit leading to early disappearance of antibody
binding sites in the *beta* portion of the hCG molecule and (b) since the
determinants of the *alpha* subunit are available for antibody binding for a
much longer time, the *alpha* subunit of the hCG molecule should be oriented
external to the *beta*. With time the orientation of the entire molecule could
change, resulting in ineffectiveness of both the subunit antisera. Hwang and
Menon (48), using a different approach of cross linking hCG to the recep-
tors, have confirmed that in hCG-receptor complex the *beta* subunit is
buried between the receptor and the *alpha* subunit. Milius et al. (49), using
subunit-specific antisera, have also concluded that the initial interaction

between hCG and the receptors takes place mainly through the *beta* subunit. According to them this interaction results in activation of the binding site in the receptor for the *alpha* subunit leading to a response. Moyle et al. (21) have developed monoclonal antibodies against the *alpha* and the *beta* subunits of hCG. They have reported five monoclonals, three of which (B101, B102, and B103) were against the *beta* subunit while two (A101 and A102) were against the *alpha* subunit. It was concluded that two of the *beta* subunit antibodies (B102 and B103) recognized the epitopes that were not covered by the receptors, while one epitope (B101) was in the receptor recognition domain of the hormone. Both the *alpha* subunit epitopes appear to be involved in binding to the receptor. The hCG *beta* antibodies we have obtained appear similar to B101 of Moyle et al. (21) while our *alpha* antiserum is different from A101 and A102 in that it is able to bind receptor-bound hCG.

In the receptor–gonadotropin–antibody interaction studies we have primarily conducted three types of experiments. Firstly it was demonstrated, for both hCG/LH as well as for FSH, that a preformed hormone–antibody complex was unable to bind the receptor (both membrane and cells). The ability of the antibody to dissociate the gonadotropin that is already complexed to the receptor depended upon several factors like the species of gonadotropin, the time for which it has been associated with the receptor and finally the titer of the antibody used. Thus, in the case of rat LH, pregnant mare serum gonadotropin, or rat and ovine FSH, addition of antiserum at any time of hormone–receptor association caused dissociation of the hormone; in the case of cells exposed to hCG for 15 min or more it is virtually impossible to dissociate the complexed hormone with antibody (19,50,51). Ovine and equine LH appear to bind the receptor much less tightly compared to hCG (51). Interestingly, ^{125}I FSH bound to intact cellular receptor is readily dissociable by antibody addition, compared to very tight binding seen when the labelled hormone is preincubated with membrane preparations (23). When the receptor preparation and antiserum are premixed before addition of ^{125}I FSH, the ability of the antibody to prevent binding of radioiodinated hormone to the receptor depends on the antibody as well as its titer. Thus similar to that observed for hCG even in the case of FSH the antiserum to the *beta* subunit is significantly less effective in blocking binding of the hormone to the receptor than is the antiserum to intact FSH.

Conclusion

The versatility and usefulness of gonadotropin antibodies in studying the action of FSH and LH at the physiological, cellular and molecular level is described in the foregoing paper. Antibodies to LH and FSH, whether they be polyclonal or monoclonal in nature, need to be characterized for specificity before being used as hormone antagonists. The cross-reactivity across species barrier which the gonadotropin antisera exhibit also makes it convenient to use a single pool of characterized antiserum for neutralizing hormonal activity in different species. Considerable inputs into our knowledge of basic aspects of reproductive endocrinology, like follicular maturation/atresia, ovulation, regulation of luteal function, maintenance of early

pregnancy and the need for FSH in regulating sperm production, have been made using this probe. More recently the LH and FSH antibodies have been used to investigate problems in hormone–receptor interaction and the response that follows as well as receptor induction and breakdown. An offshoot of the use of gonadotropin antibodies is the feasibility of using them as contraceptive vaccine for population control.

Acknowledgement

References

1. Moudgal NR & Li CH (1961) Arch. Biochem. Biophys. **93**, 122.
2. Raj HGM & Moudgal NR (1971) Ind. J. Biochem. **8**, 314.
3. Rao AJ & Moudgal NR (1970) Arch. Biochem. Biophys. **138**, 189.
4. Moudgal NR, Muralidhar K & Raj HGM (1979) in: *Methods of Radioimmunoassay* (Jaffe BM & Behrman HR, eds), p 173, Academic Press, New York.
5. Moudgal NR, Rao AJ, Maneckjee R, Muralidhar K, Mukku VR & Sheela Rani CS (1979) Rec. Prog. Horm. Res. **30**, 47.
6. Moudgal NR (1974) in: *MTP International Review of Science, Series One – Reproductive Physiology* (Greep RO, ed), vol 8, p 33, University Park Press, Baltimore.
7. Sheela Rani CS & Moudgal NR (1983) in: *Hormonal Proteins and Peptides* (Li CH, ed), vol 11, p 135.
8. Moudgal NR (1985) in: *Advances in Veterinary Science and Comparative Medicine* (Hendrix AG, ed), vol 28, p 343, Academic Press, New York.
9. Sheela Rani CS, Murty GSRC & Moudgal NR (1978) Int. J. Androl. **1**, 489.
10. Murty GSRC, Sheela Rani CS, Moudgal NR & Prasad MRN (1979) J. Reprod. Fert. Suppl. **26**, 147.
11. Raj HGM, Sairam MR, Dym M, Kotite N, French FS, Sloan C & Dy RC (1980) in: *Satellite Symp. 7th Cong. Int. Primatol. Soc. Bangalore* (Anand Kumar TC, ed), p 176, S. Karger, Basel.
12. Wickings EJ & Nieschlag E (1980) Fert. Ster. **34**, 269.
13. Moudgal NR & Rao AJ (1984) in: *Human Fertility, Health and Food* (Puett D, ed), p 73, United Nations Fund for Population Activities, New York.
14. Talwar GP, Sharma NC, Dubey SK, Salahuddin M, Das C, Ramakrishnan S, Kumar S & Hingorani V (1976) Proc. Natl. Acad. Sci., U.S.A. **73**, 218.
15. Muralidhar K, Samy TSA & Moudgal NR (1974) in: *Gonadotropins and Gonadal Function* (Moudgal NR, ed), p 169, Academic Press, New York.
16. Murthy GS & Moudgal NR, unpublished observations.
17. Midgley AR, Niswender GD, Gay VL & Reichert LE (1972) Rec. Prog. Horm. Res. **27**, 235.
18. Moudgal NR, Mukku VR, Prahalada S, Murty GSRC & Li CH (1978) Fert. Ster. **30**, 223.
19. Dighe RR & Moudgal NR (1983) Arch. Biochem. Biophys. **225**, 490.
20. Ansari AA (1985) J. Biosci. **7**, 315.
21. Moyle WR, Ehrlich PH & Canfield RE (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 2245.
22. Sairam MR (1978) Endocrine Res. Commun. **5**, 279.
23. Moudgal NR & Sairam MR, unpublished observations.
24. Rao AJ & Moudgal NR (1970) Arch. Biochem. Biophys. **138**, 189.
25. Dias JA, Huston JS & Reichert LE (1984) Endocrinology **114**, 1259.
26. Mukku VR and Moudgal NR (1979) in: *Recent Advances in Reproduction and Regulation of Fertility* (Talwar GP, ed), p 135, Elsevier/North Holland, New York.

27. Behrman HR & Armstrong DT (1969) J. Endocrinol. **52**, 419.
28. Moudgal NR, Behrman HR & Greep RO (1972) J. Endocrinol. **52**, 413.
29. Mukku VR & Moudgal NR (1975) Endocrinology **97**, 1455.
30. Dighe RR & Moudgal NR, unpublished observations.
31. Mukku VR, Murty GSRC, Srinath BR, Ramasharma K, Kotagi SG & Moudgal NR (1981) Biol. Reprod. **24**, 814.
32. Sheela Rani CS & Moudgal NR (1977) Endocrinology **101**, 1484.
33. Vidyashankar N & Moudgal NR (1984) Mol. Cell. Endocrinol. **37**, 215.
34. Sheela Rani CS, Ravindranath N & Moudgal NR (1984) Presented at the 7th Intl. Cong. of Endocrinology held at Quebec City, Canada, Abst. No. 2023.
35. Vani H & Moudgal NR, unpublished observations.
36. Dhanasekaran N, Sheela Rani CS & Moudgal NR (1984) Mol. Cell. Endocrinol. **33**, 97.
37. Moudgal NR, Moyle WR & Greep RO (1971) J. Biol. Chem. **246**, 4983.
38. Muralidhar K & Moudgal NR (1976) Biochem. J. **160**, 603.
39. Muralidhar K & Moudgal NR (1976) Biochem. J. **160**, 607.
40. Muralidhar K & Moudgal NR (1976) Biochem. J. **160**, 615.
41. Dighe RR, Muralidhar K & Moudgal NR (1979) Biochem. J. **180**, 573.
42. Moudgal NR & Li CH (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 2500.
43. Ramakrishnan S, Das C & Talwar GP (1978) Biochem. J. **176**, 599.
44. Vidyashankar N & Moudgal NR (1981) Arch. Biochem. Biophys. **209**, 241.
45. Lee CY & Takahashi M (1977) Endocrinology **101**, 869.
46. Nimrod A, Erickson GF & Ryan KJ (1976) Endocrinology **98**, 56.
47. Moyle WR, Moudgal NR & Greep RO (1971) J. Biol. Chem. **246**, 4978.
48. Hwang J & Menon KMJ (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 4667.
49. Millius RR, Midgley AR & Birken S (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 7375.
50. Bahl OP & Moyle WR (1978) in: *Receptor and Hormone Action* (O'Malley B & Birnbammer L, eds), vol 3, p 267, Academic Press, New York.
51. Sheela Rani CS & Moudgal NR (1985) Endocrinology **116**, 597.
52. Mukku VR & Moudgal NR (1976) Mol. Cell. Endocrinol. **5**, 71.
53. Sheela Rani CS & Moudgal NR (1978) Steroids **32**, 435.
54. Moudgal NR, Sairam MR & Mahoney J (1985) J. Reprod. Immunol., in press.
55. Sheela Rani CS & Moudgal NR (1978) Mol. Cell. Endocrinol. **11**, 293.
56. Sheela Rani CS & Moudgal NR (1979) Proc. Ind. Acad. Sci. **87B**, 41.