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Plasma levels of gonadotropin releasing hormone during menstrual cycle of *Macaca radiata*

N. MATHIALAGAN and A. JAGANNADHA RAO
Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

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Abstract. A sensitive radioimmunoassay for gonadotropin releasing hormone has been developed. The assay has been validated for its specificity by testing various analogues of gonadotropin releasing hormone. Analysis of plasma samples during the menstrual cycle of 4 female bonnet monkeys showed a significant increase in the immunoreactive gonadotropin releasing hormone levels during preovulatory period of the menstrual cycle.

Keywords. Gonadotropin releasing hormone; radioimmunoassay; monkey; menstrual cycle.

Introduction

Several recent studies have indicated the presence of gonadotropin releasing hormone (GnRH) like material in the placenta of human (Khodr *et al.,* 1980; Tan and Rousseau, 1982; Seeberg and Adelman, 1984), rat (Gautron *et al.,* 1981) and rabbit (Nowak *etal.,* 1984). It has also been suggested that placental GnRH has an important role in regulation of chorionic gonadotropin secretion (Khodr and Siler Khodr, 1978, 1986; Ashitaka *et al.,* 1980; Rao *et al.,* 1984a). Several of the above cited studies have been carried out using human placental explants under *in vitro* conditions.Recently, we have demonstrated (Rao and Moudgal, 1984b) that injection of GnRH by intravenous route to pregnant monkeys during early stages results in a significant increase in serum chorionic gonadotropin (CG) levels, thus providing first *in vivo* evidence for a role for GnRH in regulation of CG secretion in primates. Although several reports are available on the plasma levels of GnRH during menstrual cycle of the human female, practically no information is available on the GnRH levels during menstrual cycle or pregnancy in non-human primates. In view of this and as an extension of our *in vivo* study, we have initiated detailed studies on the role of GnRH during menstrual cycle and early pregnancy in bonnet monkeys. The present paper describes the development and application of radioimmunoassay (RIA) of GnRH to monitor the plasma levels of GnRH during menstrual cycle of bonnet monkeys, *Macaca radiate*

Abbreviations used: GnRH, Gonadotropin releasing hormone; CG, chorionic gonadotropin; RIA, radioimmunoasay; GPBS, Gelatin-phosphate buffered saline 0·01 M, pH 7·4.

Materials and methods

GnRH was obtained from Ayerst and Wyeth laboratories. New York, USA; other analogous used in the study were gifts from the following sources: Buserelin from Dr. J.Sandow, Hoechst, Federal Republic of Germany; GnRH-proethyla mide, D-Trp^6-GnRH and D-Lys^6-GnRH from NIH, USA; D-Phe^6-GnRH, Trp^7-Leu^8-GnRH and D-Phe^6-Trp^7-Leu^8-GnRH from Dr. G. Keri, Hungary. Antiserum to GnRH was raised in adult male rabbits according to the procedure of Koch *et al.* (1973). The antiserum was characterized by checking its binding to iodinated GnRH and its cross reactivity with several analogues of GnRH. Iodination of GnRH was done using Iodogen method. The separation of iodinated GnRH from free iodine was achieved by fractionating the reaction mixture on G-25 Sephadex column (45 × 0·9 cm) equilibrated with 0·01 M phosphate buffered saline containing 0·1 % gelatin-phosphate buffered saline 0·01 M, pH 7·4 (GPBS). The specific activity of iodinated GnRH ranged from 200–250 μCi/μmoI. All other reagents used in the study were analytical grade, procured from local sources.

RIA of GnRH

Varying quantities of GnRH (1-500 pg in 100 μl) were added to 3·0 ml glass tubes containing 100 μl of rabbit antiserum to GnRH (final dilution of 1:3,000) followed by 100 μl of [^{12S}I]-GnRH (30,000 cpm). The total incubation volume was 300 μl and the incubation was carried out at 4°C for 12 h. Separation of the free label from bound was achieved according to the method of Hichens *et al.* (1974) by addition of 500 μl of Dextran coated charcoal (0·3% dextran T 70 and 3% activated charcoal in 0·01 M PBS, pH 7·4) followed by centrifugation at 3000 *g* for 15 min at 4°C. Four hundred μl aliquot of the supernatant was counted in a LKB gamma counter.

Animals

Regularly cycling adult female monkeys weighing 5-6 kg were used in the study. The husbandary of the monkey colony was as described earlier (Prahlada *et al.,* 1975). The day on which vaginal bleeding was first seen was considered as day one of the menstrual cycle. The average length of the menstrual cycle of monkeys in the colony is 28 ± 2 days (n = 30). Blood samples were collected from unanesthetised animals between 11.00-11.30 a.m. on every day using heparinised vaccutainer tubes and plasma was separated by centrifuging at 1000 *g* for 5 min at room temperature.

Extraction of plasma

The clear plasma (about 1·5—2·5 ml) was transfered to tubes containing 10·0 ml of methanol, vortexed for 2 min and centrifuged at 10,000 *g* for 5 min. The supernatant was saved and the pellet was re-extracted with 100 ml methanol for 2 min and the two supernatants were pooled and evaporated to dryness at 55°C in a waterbath. The residue was reconstituted in 0·5 ml of GPBS and suitable aliquots were used for the assay.

Recovery of GnRH during extraction was ascertained by following the recovery of added [^{125}I] -GnRH. The recovery ranged from 78–84% (n = 9).

Validation of the GnRH assay

The RIA of GnRH was validated by assaying increasing quantities of plasma extract in the assay and checking for parallelism in the displacement curve obtained. The procedure employed to extract GnRH from the plasma was validated by monitoring the increase in GnRH levels following a bolus injection of GnRH. Two male monkeys were injected with a dose of 100 μg GnRH by intravenous route *via* femoral vein and blood samples were collected at specified intervals and the plasma obtained were extracted as described earlier and assayed for GnRH, following reconstitution.

Results

A standard inhibition plot for GnRH is presented in figure 1 and it can be seen that the assay range is from 10–250 pg of GnRH. The lowest concentration of GnRH which can be detected with certainity was 15 pg. The antiserum seems to be highly specific for the native GnRH sequence as all analogues tested showed less than 1% cross reactivity at 50% displacement of the assay. Plasma used for parallel displacement was a concentrated extract of plasma from female monkeys during various phases of menstrual cycle. The extracted plasma showed a parallel displacement curve (figure 1) in the GnRH-RIA similar to that of standard. The intra and inter assay variations were 8·5% and 10·8%, respectively.

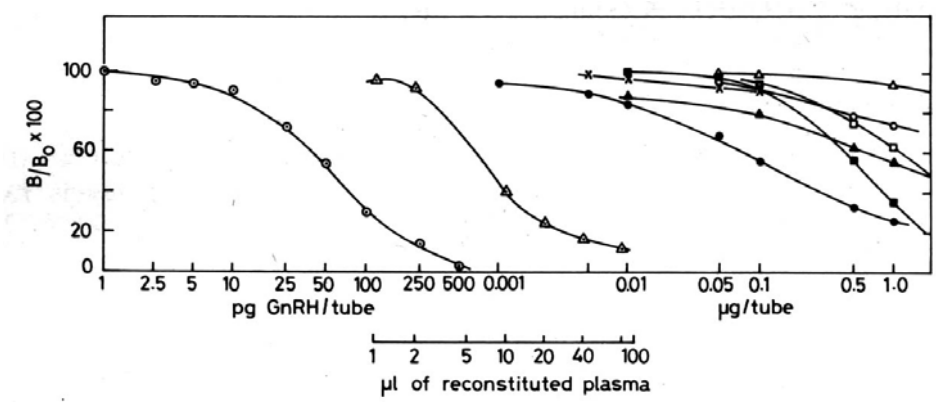

Figure 1. Standard inhibition plot for GnRH-RIA. (☉) ., Cross reaction for analogues of GnRH; (●), Buserelin; (□), D-Trp^6-GnRH; (▲). D-Lys^6-GnRH; (O). D-Phe^6-GnRH; (Δ). Trp^7-Leu^8-GnRH; (×). D-Phe^6-Trp^7-Leu^8-GnRH; (■).GnRH-proethylamide; (△) parallel displacement by plasma extract.

Validation of the GnRH-RIA

The results presented in figure 2 clearly show that following injection of a bolus dose of GnRH, there is a significant increase in the plasma levels of GnRH. The preinjection values are 5-8 pg in monkey No. 456 and 10–20 pg in monkey No. 726 and following injection of GnRH, by 5 min the plasma value increased 5–6 fold from 0 min value in both monkeys, indicating that only GnRH is quantitated by RIA and the observed GnRH in RIA is not due to any non-specific interference.

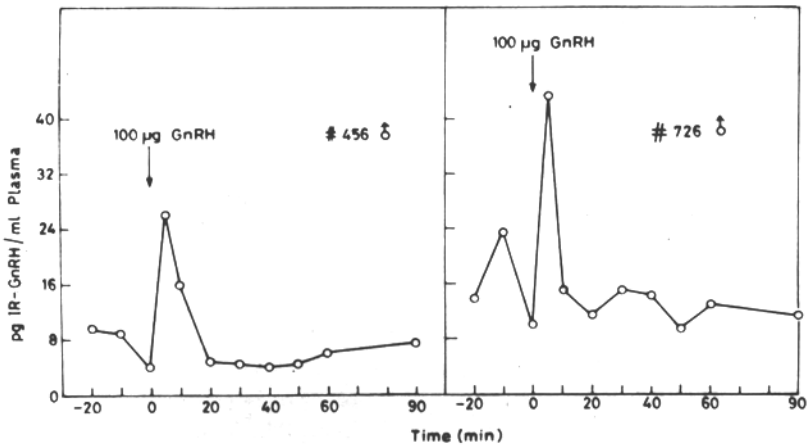

Figure 2. Plasma immunoreactive GnRH levels in adult male monkeys following injection of 100 μg of GnRH by intravenous route.

The increase observed however does not account for all the GnRH injected and it is known that more than 80% of the injected GnRH is cleared from the plasma within 2 min (Elkind-Hirsch *et al.,* 1982) in the case of humans. It can also be seen that the values returned to basal levels by 10 min and the fall in plasma levels is very rapid indicating that GnRH is cleared very fast in monkeys as in the case of humans.

Plasma GnRH levels during menstrual cycle in the monkey

The profiles of plasma immunoreactive GnRH levels during the menstrual cycle in the 4 female bonnet monkeys is shown in figure 3. The levels ranged from

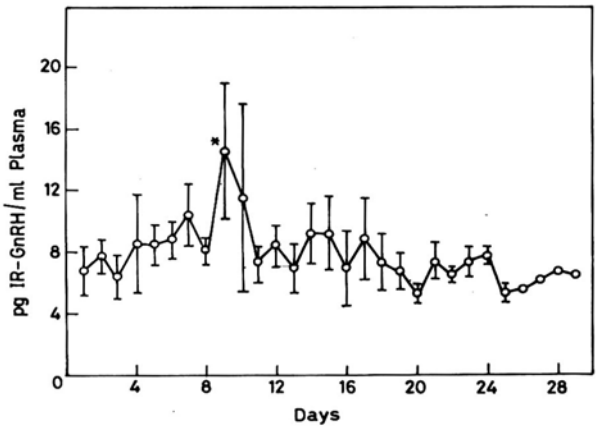

Figure 3. Plasma immunoreactive levels of GnRH during menstrual cycle in adult female monkeys. Mean ± SEM, n = 4

* Significantly different from day 1 value $P<0{\cdot}05$.

7-10 pg/ml during follicular phase, following which a significant (P<(0·05) elevation of immunoreactive GnRH was seen by 9th day and the level decreased to basal levels by 11th day after which there was no significant variation.

Discussion

In the present study a specific RIA has been developed for monitoring plasma GnRH levels. The RIA has been validated for specificity and by monitoring the levels of GnRH following a bolus injection of GnRH, we have established the authenticity of the GnRH quantitated by RIA. The endogenous immunoreactive GnRH appear to be indistinguishable from the synthetic decapeptide used as a standard and the inhibition plot obtained by using various quantities of plasma extract was completely parallel to the standard curve. However, the discrepancy in the quantity of injected GnRH and the peak values obtained could be due to the interval in sampling. It may be pertinent to point out that in the studies of Elkind-Hirsch *et al.* (1982) the maximum values obtained at 1 min sampling was 515 pg, following 100 μg of GnRH injection and this also does not account for all the GnRH injected.

The observation that peak immunoreactive GnRH levels occur by 9th day of menstrual cycle in the bonnet monkey is in agreement with the report of Elkind-Hirsch *et al.* (1982) that peak levels of immunoreactive GnRH occur during preovulatory phase of human menstrual cycle. It is known that in the bonnet monkey serum estradiol–17 β surge occurs around day 9-10 and preovulatory surge of lutinising hormone (LH) occurs within 24 h of this surge (Rao *et al.,* 1984c). In this connection, the observation made in the present study, that the plasma GnRH also reaches peak value in bonnet monkeys by 10th day of menstrual cycle is of considerable significance and interest. It is possible that this increase in GnRH in levels may also have a role in the regulation of the release of gonadotropins during preovulatory surge in non-human primates also (Crowley *et al.,* 1985)

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References

Ashitaka, Y., Nishimura. R., Takemore, M. and Tojo. S. (1980) in *Chorionic gonadotropin* (ed. Sheldon J. Segal) (New York: Plenum) p. 147.

Crowley. W. F. Jr., Filicori. M., Spratt. D. I. and Santoro. N. F. (1985) *Recent Prog. Hrom. Res.,* **41,** 473.

Elkind-Hirsch. K., Ravniker. V., Schiff. I., Tulchinsky, D. and Ryan. K. J. (1982)*J. Clin. Endocrinol. Metab.,* **54,** 602.

Gautron, J. P., Pattou, E. and Kordon.C. (1981) *Mol. Cell. Endocrinol.,* **24,** 1.

Hichens, M., Gale, P. H. and Schawan, H. (1974) in *Methods in Radioimmunoassay* (eds M. B. Jaffe and R. H. Behrman) (New York: Academic Press Inc.) p. 45.

Khodr, G. S. and Siler-Khodr, T. M. (1978) *Fertil. Steril.,* **30,** 301.

Khodr, G. S. and Siler-Khodr. T. M. (1980) *Science.* **207,** 315.

Koch, Y., Wilchek, M., Fridkin, M., Chobsieng, P., Zor, U. and Lindner. H. R. (1973) *Biochem. Biophys. Res. Commun.,* **55,** 616.

Nowak, R. A., Wiseman, B. S. and Bahr, J. M. (1984) *Biol. Reprod.,* **31,** 67.

Prahlada, S., Mukku, V. R., Rao. A. J. and Moudgal, N. R. (1975) *Contraception,* **12,** 137.

Rao, A. J., Mathialagan, N., Kotagi, S. G. and Moudgal, N. R. (1984a) *J. Biosci.,* **6,** 97.

Rao. A. J. and Moudgal, N. R. (1984b) *IRCS Med. Sci.,* **12,** 1105.

Rao, A. J., Kotagi, S. G. and Moudgal, N. R. (1984c) *J. Reprod. Fertil.,* **70,** 449.

Seeberg, P. H. and Adelman, J. P. (1984) *Nature (London),* **311,** 666.

Siler-Khodr, T. M., Khodr, G. S., Valenzeula, G. and Rhode, J. (1986) *Biol. Reprod.,* **34,** 245.

Tan, L. and Rousseau, P. (1982) *Biochem. Biophys. Res. Commun.,* **109,** 1061.