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EFFECT OF LHRH AGONISTS AND ANTAGONISTS IN MALE AND FEMALE BONNET MONKEYS (*MACACA RADIATA*)

A. JAGANNADHA RAO, R. CHAKRABORTI, S. G. KOTAGI, N. RAVINDRANATH and N. R. MOUDGAL
Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, India

Summary—A few analogues of LHRH have been tested in the adult bonnet monkeys using change in serum testosterone following LHRH injection as a parameter of response to LHRH. Of the four analogues tested in male monkeys, Buserelin was found to be the most potent one in increasing serum testosterone levels. Injection of the LHRH antagonist at 1600 h resulted in the abolition of the characteristic nocturnal surge of testosterone observed in adult bonnet monkeys maintained under regulated light conditions. Following administration of LHRH a/s during early pregnancy, serum chorionic gonadotropin levels decreased though the course of pregnancy was not affected. These results suggest that bonnet monkey can be successfully employed to test LHRH analogues.

INTRODUCTION

Ever since the elucidation of the structure of Luteinizing hormone releasing hormone (LHRH), several analogues with agonistic and antagonistic properties have been synthesized. These have been tested in a variety of species using both *in vitro* as well as *in vivo* systems. *In vivo* studies have been mostly carried out in small animals such as rats, rabbits, mice and guinea pigs. Attempts to evaluate the analogues in higher non-human primates have been not very successful as several species were found to respond poorly to LHRH and its analogues, particularly, the bonnet monkey and rhesus [1–5]. However, since the publication of these reports, several newer analogues have been synthesized, some of which were found to be effective in stumptailed monkeys and chimpanzees [6, 7]. In the present study we have examined the effect of some of the LHRH analogues in the South Indian bonnet monkey, *Macaca radiata*.

EXPERIMENTAL

The LHRH and its analogues used in the study were obtained as gift from the following agencies. Synthetic LHRH from Ayerst and Wyeth Laboratories, U.S.A.; synthetic LHRH (amide form, lot No. 21-103-DH), LHRH superagonist Trp^6 $Des\text{-}Gly^{10}$ proline ethyl amide and LHRH antagonist (CDB 2085-A) from NIH, U.S.A. Buserelin (Hoe 766) from Hoechst Pharmaceuticals, West Germany. Antiserum to ovine luteinizing hormone (LH a/s) used in the study was raised in male monkeys and characterized as described earlier [8]. Serum testosterone was measured by a method standardised in the laboratory [9]. The details of radioimmunoassay of monkey chorionic gonadotropin as well as husbandry, maintenance, breeding, blood sampling of monkeys has been described in earlier publication [10]. LHRH antiserum used in the study was a gift from Dr G. P. Talwar, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi.

RESULTS

Effect of LHRH and its analogues on serum testosterone levels in the adult male bonnet monkey

It has been demonstrated by Arslan *et al.*[11] that following the increase in serum LH in response to LHRH administration, there is also an increase in serum testosterone in the rhesus monkey. In view of the ease with which serum testosterone can be monitored, we have considered the change in serum testosterone following LHRH injection as a parameter of response to LHRH. The effects of injection of various analogues of LHRH on serum testosterone levels is presented in Table 1. All injections were given by intravenous route after collecting a control sample. It can be seen that all the analogues were effective in increasing serum testosterone levels by 30 min. However, of the four analogues tested Buserelin was the most effective as injection of as little as one μg resulted in a 20-fold increase in serum testosterone. As change in serum testosterone was considered as a parameter of response to LHRH, it was necessary to establish that the observed effect of LHRH was mediated via LH release. In order to accomplish this, LH a/s was injected just before the injection of LHRH. The data presented in Table 2

Table 1. Effect of LHRH and its analogues on serum testosterone levels in adult male bonnet monkeys

Type of analogue injected	Dose μg	Serum testosterone ng/ml at time 0 min	Serum testosterone ng/ml at time +30 min
Synthetic LHRH	10	2.2 ± 0.2	11.2 ± 2.4
D Trp^6-des-gly^{10} proethylamide	5	2.6 ± 0.7	18.2 ± 3.1
Lys^6 LHRH	10	3.09 ± 1.0	12.6 ± 0.89
Buserilin	1	0.65 ± 0.09	13.1 ± 1.92

Number of animals in each group = 4.
Each value Mean ± SEM
All injections were given by i.v. route at 1000 h.

Table 2. Evidence to suggest the involvement of LH in mediating the action of LHRH

Treatment	Serum testosterone ng/ml Mean ± SE Before injection	Serum testosterone ng/ml Mean ± SE 60 min after injection
LHRH alone (5 μg D-TRP^6-des-gly^{10} Pro ethylamide)	1.6 ± 0.19	20.3 ± 5.6
LHRH + LH a/s*	1.4 ± 0.14	5.2 ± 2.3

*5 μg of LHRH (D-Trp^6-des-gly^{10} Pro ethylamide + 2 ml of OLH a/s raised in monkeys injected by i.v. route prior to LHRH. n per group = 3.

shows that injection of LH a/s resulted in complete abolition of the increase in serum testosterone, suggesting that the increase in serum testosterone is due to LH released in response to LHRH.

Though several antagonists have been found to be effective in lower experimental animals, studies in monkeys have not given equivocal results. We have evaluated one of the antagonists (CDB 2085-A) using the ability to abolish the characteristic nocturnal surge of testosterone as a parameter. Earlier studies from our laboratory have demonstrated that the adult male bonnet monkeys maintained under regulated light condition exhibit a surge of testosterone around 2200 h [12]. Studies have also shown that this surge could be abolished by injection of LH a/s [9]. The results of the effect of injection of antagonist are presented in Table 3. It can be seen that on the day prior to injection of antagonist, the animals demonstrated a clear surge of testosterone at 2200 h. However, following injection of antagonist at 1600 h on the following day, the nocturnal surge of serum testosterone was completely abolished.

Effect of LHRH antiserum on serum chorionic gonadotropin levels in pregnant monkeys

Several recent reports have established the presence of an LHRH like material in human placenta which is immunologically identical to hypothalamic LHRH [13, 14]. *In vitro* studies using human placental cultures have revealed that addition of synthetic LHRH results in an increase in CG secreted into medium [15, 16]. Based on these studies it has been suggested that placental LHRH may have a role in regulation of secretion of CG during pregnancy in primates. Our own *in vivo* studies [17] using pregnant bonnet monkey as a model as well as others [18] have shown that injection of LHRH by i.v. route results in an increase in serum chorionic gonadotrophin levels within 60 min also lend support to such a suggestion. Recently we have extended *in vivo* studies using antiserum to LHRH. Antiserum to LHRH was injected by i.v. route to pregnant monkeys on any one day between 16–29 day of pregnancy after collecting a pre-injection sample. Serum chorionic gonadotropin was estimated using a specific radioimmunoassay in samples collected at various time points up to 24 h. The data presented in Table 4 shows that following injection of LHRH a/s there is a decrease in serum CG levels, though the course of pregnancy was not affected.

Table 3. Effect of injection of LHRH antagonist on nocturnal surge of testosterone in adult bonnet monkeys

Serum testosterone ng/ml at: Day previous to injection 1000 h	Day previous to injection 2200 h	Day of injection* 1000 h	Day of injection* 2200 h
2.9 ± 0.95	23.4 ± 3.3	3.2 ± 1.1	1.53 ± 0.46

*The group of monkeys received at 1600 h 1 mg of the antagonist (CDB-2085-A) by i.v. route.
n = 4 monkeys per group; all values Mean ± SEM.

Table 4. Effect of injecting LHRH antiserum on serum CG levels in pregnant monkeys

Monkey no.	Volume of a/s injected (ml)	% Decrease in serum CG at 4 h	8 h	12 h	24 h
408 (16)	1.5	76	80	85	85
7 (29)	0.5	13	17	47	29
6 (25)	1.0	60	71	61	57

The numbers in parenthesis indicate the day of pregnancy on which a/s was injected. All injections were given by i.v. route. Pre-injection value was considered as 100% and the change expressed as % of it.

DISCUSSION

The classical observation that the agonists of LHRH have paradoxical effects, have prompted several investigators to explore the possibility of using the LHRH analogues as antifertility agents. Several agonists and antagonists have been tested in lower experimental animals as well as in non-human primates. It has been realised from these studies that the choice of the experimental animal is one of the important factors to be considered in evaluating the activity of LHRH analogues. Before the availability of the super agonist (D-Trp^6-Des-Gly^{10} proline ethyl amide) and Buserelin, based on the studies with other less potent analogues of LHRH, it was concluded that the response to LHRH is poor in many non-human primate species. It was suggested that the refractoriness of the monkey to exogenously administered LHRH is attributable to its rapid degradation. The results of the present study also lend support to such a suggestion. Of the four analogues tested, the synthetic LHRH with no modification, is the least potent one and other analogues with substitution at position 6 which is known to make LHRH resistant to enzymatic degradation as well as increased affinity for receptor cells are relatively more potent. In view of the several reports that LHRH may have a direct action at the gonadal level, our observation that the increase in serum testosterone can be abolished by prior injection of LH antiserum is of considerable significance. This clearly establishes that the increase in serum testosterone is the result of action of LH on testis and not due to direct action of LHRH. Though injection of LHRH a/s was effective in decreasing serum CG, it was not effective in terminating pregnancy and this could be due to insufficient neutralisation or the period of injection. Our studies also show that the nocturnal surge of testosterone, a characteristic feature of bonnet monkey, can be abolished with the antagonist. The involvement of LH in regulation of this surge has been demonstrated in the present study as well as earlier studies [13]. In contrast to the complexity of the female monkey model used (either during menstrual cycle or pregnancy requiring experimentation over a prolonged period), the proposed male bonnet monkey model is quite simple and the analogues can be evaluated within a day, the monkeys being available for reuse within 3–4 days. In summary, the adult male bonnet monkey can be successfully employed to quickly screen the activity of agonists and antagonists of LHRH.

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