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SPECIES DIFFERENCES IN THE SENSITIVITY TO GnRH ANALOGS

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Summary—The effects of several GnRH agonists and antagonists with high biological activity, have been investigated in rats, mice, rabbits and monkeys. Striking differences exist in the response of different species to the antigonadal and antipituitary effects of these peptides. Of all the animals studied, the rat is the most sensitive. The magnitude of the response to GnRH agonists seems to depend on the sensitivity of the pituitary and the presence of GnRH receptors in the target organs. Findings from animal models require careful interpretation before predictions can be made regarding their possible effects in the human.

INTRODUCTION

Since the elucidation of the structure of GnRH, many analogs with agonistic or antagonistic action have been synthesized [1]. Antigonadal and anti-fertility effects of potent agonists have been extensively studied. Potent antagonists have been synthesized more recently and only limited investigations have been carried out [2, 3].

GnRH analogs are useful in the treatment of a variety of disorders associated with gonadal dysfunction and they also provide a new approach for contraception in both men and women [4, 5]. In addition to the effects attributable to the action of these peptides on the pituitary, they have been shown to have extra-pituitary effects [6–12]. Because of the great differences seen in the sensitivity of various species to GnRH analogs, the choice of the species used for experiments is critical for the extrapolation of the results obtained.

This presentation reviews differences in the sensitivity of rats, mice, rabbits and rhesus monkeys to the pituitary-mediated and extra-pituitary effects of several GnRH analogs. The GnRH analogs used in these studies are presented in Table 1.

EFFECTS OF GnRH AGONISTS

Pituitary-mediated effects

We have evaluated the effects of D-His or D-Trp on testicular function of adult rats, mice and monkeys. A single dose of an agonist caused a rapid release of LH and FSH from the pituitary and sharp increases in serum LH, FSH and testosterone levels in all three species [13–15]. In contrast, the three species responded differently to repeated daily treatment with GnRH agonists. While rats showed a marked decrease in serum testosterone concentrations, associated with a time-dependent decline in testicular LH receptor levels after 6 days of treatment, no significant effect on either testosterone or testicular LH receptors was seen in mice (Fig. 1). The response in monkeys to chronic agonist administration was more similar to that of mice. In spite of a marked decrease in LH and FSH response, the effect on testosterone was less evident in rhesus monkeys, treated daily with relatively high doses (100 μg/day) of D-His for several months. Similar observations were reported by Akhtar *et al.*[16] and Resko *et al.*[17] following daily administration of 25 to 100 μg of Buserelin (Hoe 766) another potent agonist. However, subsequent studies indicated, that the mode of administration is critical. Continuous infusion of Hoe 766 induced a dramatic decline in testicular function [18]. Overall, these results suggest that, compared to rats, mice and monkeys are less sensitive to the antitesticular effects of GnRH agonists. In mice and rhesus monkeys the pituitary is more susceptible than the testes to desensitization by chronic agonist treatment. In addition, there are differences among the species in the susceptibility to the direct antitesticular effects of GnRH agonists.

Direct antigonadal effects

GnRH and its agonists have been shown to exert direct, extrapituitary actions on various target organs in male and female rats [9, 19, 20]. In the testes, GnRH-agonists act on androgen production and LH receptor levels of immature and adult hypophy-

Table 1. GnRH analogs

Agonists
D-His: [(imBzl)-D-His^6,Pro^9-NEt]-GnRH
D-Trp: [(D-Trp^6,Pro^9-NEt]-GnRH
Hoe 766: [D-Ser(TBu)^6,Pro^9-NEt]-GnRH
Antagonists
A_1: [Ac-D-NAL(2)^1, 4F-D-Phe^2, D-Trp^3, D-Arg^6]-GnRH
A_2: [Ac-Δ Pro^1, 4F-D-Phe^2, D-Trp^{3,6}]-GnRH
A_3: [Ac-Δ Pro^1, 4F-D-Phe^2, D-NAL(2)^{3,6}]-GnRH

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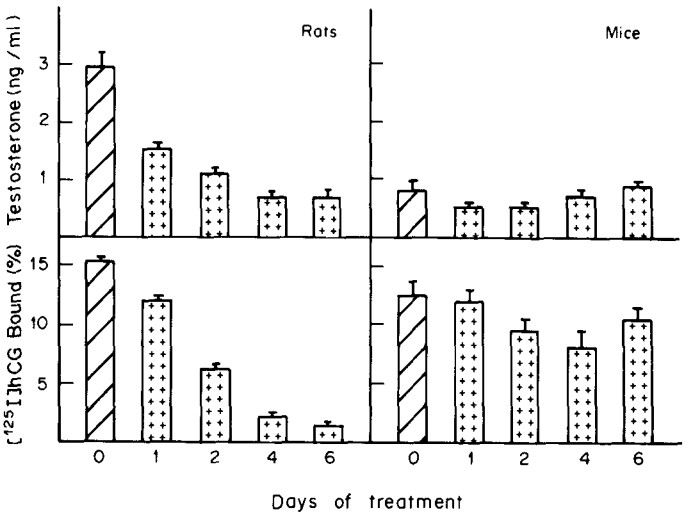

Fig. 1. Effects of D-His on serum testosterone levels and testicular LH receptor concentrations ([^{125}I])hCG binding capacity) in intact rats and mice. Groups of 6 animals were given 1 μg D-His daily for 0–6 days and killed on the day after the last injection. Means and SEs are shown (reproduced from Wang *et al.*, 1983). Copyright, 1983, The Endocrine Society.

sectomized rats. We have compared the direct effects of two GnRH-agonists in hypophysectomized mice with those in rats. Both species, following pretreatment with FSH, responded to hCG treatment with a marked increase in serum testosterone. When D-His was administered along with FSH, the hCG-induced steroidogenic response of rats was completely inhibited, while that of mice was unchanged (Fig. 2), suggesting that mice are insensitive to direct antigonadal effects of GnRH agonists. In a similar experiment, the *in vitro* testosterone production by decapsulated testes from hypophysectomized animals pretreated with FSH or FSH plus D-His for 5 days was also investigated. Basal as well as hCG-stimulated testosterone production was inhibited by 85% in D-His treated rats, but was unaffected in D-His treated mice. Similarly, D-Trp also exerted antitesticular effects in hypophysectomized rats, but was without effect in hypophysectomized mice. These results are consistent with the observation by Hunter *et al.*[21] and by us that receptors for GnRH can be demonstrated in rat Leydig cells but not in mouse Leydig cells (Fig. 3).

In rhesus monkeys, as in mice, testicular receptors for GnRH could not be detected [22]. This may explain our observation that in rhesus monkeys, even after 40 weeks of daily agonist treatment the testicular steroidogenic response to a challenge with 50 I.U. of hCG was unimpaired (Fig. 4).

Species differences in sensitivity to agonists have been reported by several investigators. In contrast to the dog and rat, the rhesus monkey and mouse were relatively resistant to the antitesticular effects of GnRH agonists [23–25]. Man, on the other hand, seems more sensitive to the effects of agonists [26–28]. The differences among the various species in sus-

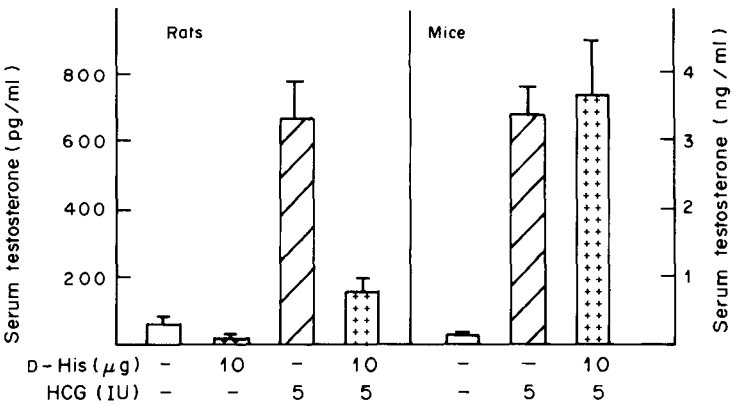

Fig. 2. Differential effects of D-His on serum testosterone levels in hypophysectomized rats and mice. Twenty-five day old hypophysectomized rats and mice were given oFSH (50 μg) with or without D-His (10 μg) daily for 5 days. On day 6, they were given hCG (5 IU, i.p.), killed 2 h later, and the serum testosterone levels were measured. Each bar represents the mean $\pm$ SE of 6–10 animals (reproduced from Wang *et al.*, 1983). Copyright, 1983, The Endocrine Society.

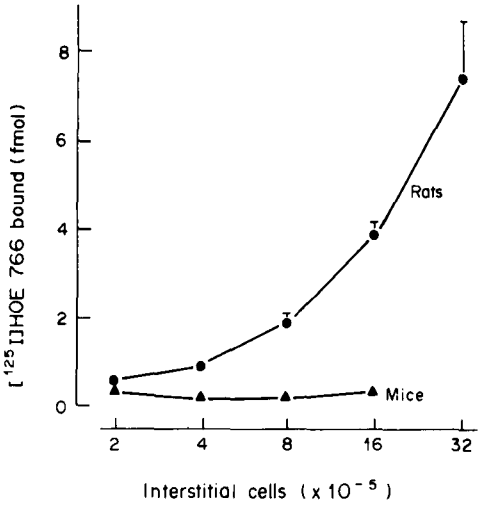

Fig. 3. Specific binding of [^{125}I]Hoe 766 to rat and mouse interstitial cells isolated by incubation of decapsulated testes with collagenase. Means and SEs of triplicate determinations are shown (reproduced from Wang *et al.*, 1983). Copyright, 1983, The Endocrine Society.

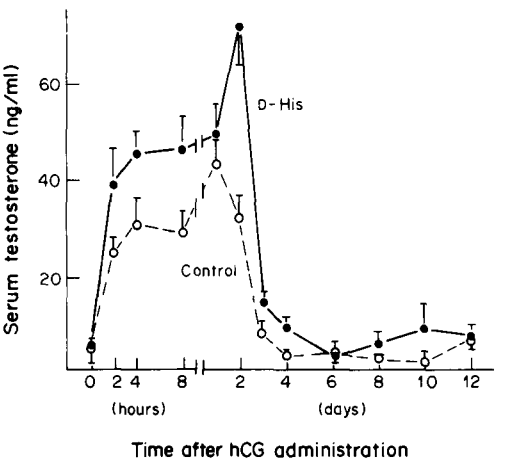

Fig. 4. Changes in serum testosterone concentrations after the administration of hCG (50 IU, s.c.) to control and D-His-treated rhesus monkeys, hCG was given 24 h after the last D-His injection. Values are mean ± SE for 6 animals (reproduced from Sundaram *et al.*, *J. Reprod. Fert.* 1984).

ceptibility to the direct, non-pituitary mediated antigonadal effects are most likely due to differences in the gonadal concentration of GnRH receptors.

The above mentioned species differences in sensitivity to the antitesticular effects of GnRH agonists prompted us to examine the effects of GnRH antagonists in various species.

EFFECTS OF GnRH ANTAGONISTS:

Antitesticular effects

The antigonadal effects of the potent GnRH antagonist Ac-D-NAL(2)[1], 4F-D-Phe[2], D-Trp[3], D-Arg[6]-GnRH (A_1) was investigated in mice, rats, rabbits and rhesus monkeys [4]. Daily treatment with A_1 for 15 days decreased serum LH and testosterone levels and the weights of seminal vesicles and ventral prostate of rats in a dose-related manner (Table 2). In contrast to the effects in rats, A_1 did not exhibit antigonadal effects in rabbits (Table 3). Histologically, the testes and epididymides showed extensive degeneration in rats, but not in rabbits. These findings were consistent with the observation that mating behavior and fertility were significantly impaired in rats but not in rabbits. The responses of rats and mice to A_1 also showed marked differences. Following A_1 administration for 5 days, rats showed significant reductions in serum testosterone secretion and *in vitro* testosterone production by testes, while no decrease was found in mice (Fig. 5).

Acute antagonist treatment in rats, mice and rabbits showed that A_1 was biologically active in all three species. Serum testosterone levels decreased rapidly with a nadir in testosterone levels 4 h after the administration of A_1 (Fig. 6). In rats, the low levels were maintained for up to 24 h, while in mice and rabbits serum testosterone concentrations started to rise after 4–8 h and reached preinjection levels by 24 h. Male rhesus monkeys reacted similarly to mice and rabbits and showed a transient decrease in serum testosterone following a single injection of A_1.

In summary, these results indicate that there are considerable differences in the sensitivity of several species to A_1. In rats a single dose resulted in suppression of plasma testosterone levels for 24 h, while in rabbits, mice and monkeys similar doses did not result in prolonged suppression. As mentioned earlier, mice and monkeys differ from rats also in their sensitivity to the antigonadal effects of GnRH agonists.

Table 2. Organ weights and hormone levels in rats treated with A_1 for 15 days and killed on day 16

	Weights				Serum Hormones†	
Daily dose (μg/kg B.wt)	Body (g)	Testes (g)	Seminal vesicles (mg)	Ventral prostate (mg)	Testosterone (ng/ml)	LH (ng/ml)
0	373 ± 10*	3.42 ± 0.1	697 ± 43	589 ± 53	1.9 ± 0.3	29.6 ± 4.2
50	368 ± 4	3.58 ± 0.08	585 ± 32	589 ± 68	2.3 ± 0.6	34.7 ± 4.6
250	381 ± 11	3.59 ± 0.09	504 ± 24‡	450 ± 33†	1.5 ± 0.1	15.3 ± 1.7‡
1250	379 ± 14	2.18 ± 0.14‡	192 ± 30‡	127 ± 23‡	0.3 ± 0.02†	ND

*Results shown are mean ± SE.
†Hormone concentrations measured 24 h after the last treatment.
‡$P < 0.01$; †$P < 0.05$; ND = below the limit of sensitivity.
$n = 10$ for all groups.

Table 3. Organ weights and serum testosterone levels in rabbits treated with A_1 for 15 days and killed on day 16

Daily dose (μg/kg BW)	Weights: Body (kg)	Testes (g)	Seminal vesicles (mg)	Ventral prostate (mg)	Serum hormones: Testosterone‡ (ng/ml)
0	3.45 ± 0.09*	5.69 ± 0.31	627 ± 39†	710 ± 100	1.7 ± 0.6
50	3.65 ± 0.11	5.14 ± 0.42	695 ± 5	1050 ± 100	2.0 ± 0.2
250	3.48 ± 0.12	5.63 ± 0.40	581 ± 63	950 ± 140	1.3 ± 0.6
1250	3.43 ± 0.16	6.0 ± 0.25	456 ± 36	1390 ± 170§	1.6 ± 0.3

*Results shown are mean ± SE.
†Tissues from 4 animals only.
‡Serum testosterone measured 24 h after last treatment.
§$P < 0.05$.
$n = 6$ for all groups.

Even though in the rat gonads both GnRH agonists and antagonists bind to the same GnRH receptors, antagonists have not been shown to have direct inhibitory action. It is therefore likely that the antitesticular action of GnRH antagonist in all species is mediated via the pituitary and that differences among species are due to differences in the sensitivity of their pituitaries to the antagonist. Differences among species in the rate of inactivation of the antagonist may also play a role.

Antiovulatory and antipituitary effects

Our findings in male animals prompted us to examine whether species differences in sensitivity to

Table 4. Ovulation inhibition in rats and rabbits with the GnRH antagonist A_1

Group	Dose μg/animal	No. animals ovulating/ No. animals treated
Cycling rats*	0	7/9
	0.2	7/10
	1	0/9
	5	0/9
Rabbits†	0	12/14
	100	4/6
	500	3/11

* Injected s.c. at 12 noon of proestrus and examined at 9 a.m. of estrus.
† Injected 0.5 h before mating. Pregnancy confirmed by serum progesterone levels and delivery.

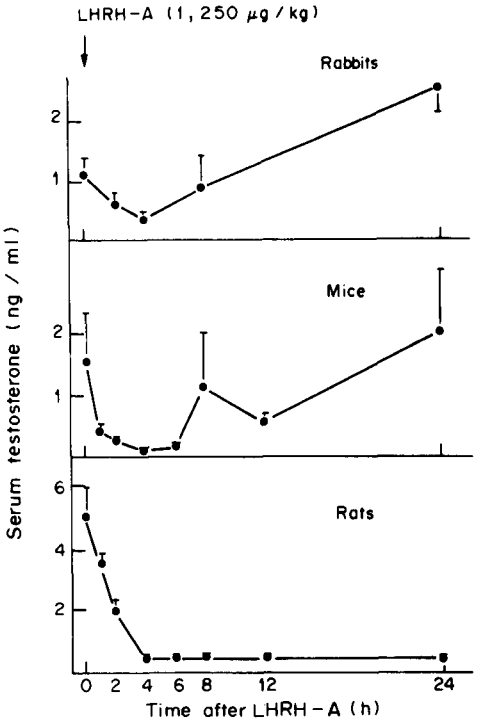

Fig. 6. Changes in mean serum testosterone levels (mean ± SE) in rats, mice and rabbits after the administration of a single dose of A_1 (1250 μg/kg s.c.). Each point represents data from 5–7 animals for rats and mice. $N = 4$ for rabbits.

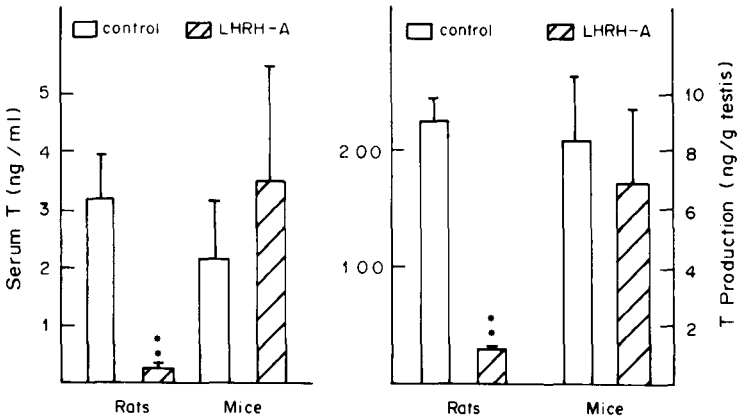

Fig. 5. Differential effects of antagonist A_1 (1450 μg/kg) on serum testosterone levels and *in vitro* testosterone production by testes from rats and mice following daily s.c. Each bar represents the mean ± SE of 6–11 animals or testes (reproduced from Sundaram *et al.*, *Contraception* 1984).

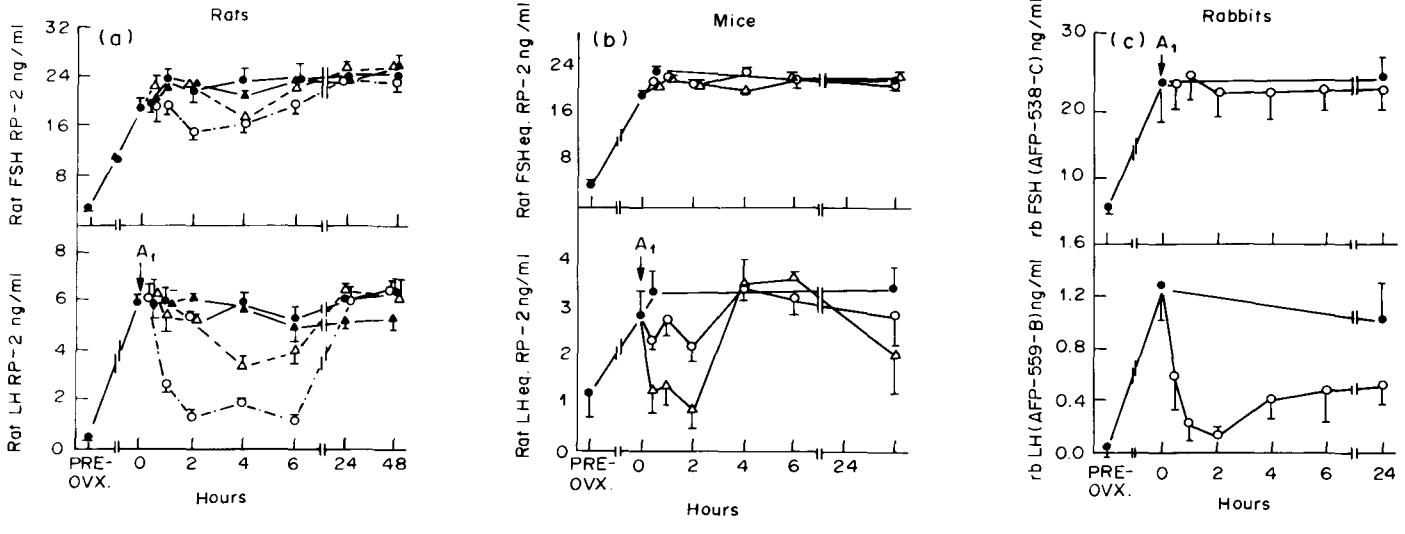

Fig. 7. Plasma LH and FSH levels (means ± SE, $N = 5$–7) in ovariectomized animals at different times following treatment with A_1 (LHRH-1) dissolved in 40% propylene glycol in saline and administered s.c.: a. Rats received 0 (closed circles), 0.1 μg/kg (closed diamonds), 1 μg/kg (open diamonds) or 10 μg/kg (closed circles). b. Mice received 0 (closed circles), 10 μg/kg (open circles) or 100 μg/kg (triangles). c. Rabbits received 0 (closed circles) or 100 μg/kg (open circles).

antigonadal or antipituitary effects of antagonists were also present in females. Suppression of ovulation is the method most widely used to evaluate the biological activity of GnRH antagonist [29]. We have investigated the capability of A_1 to inhibit ovulation in rats and rabbits and its effects on plasma LH levels in ovariectomized rats, rabbits and mice [30]. Dose–response studies showed that 1 μg (4 μg/kg) of A_1 was sufficient to inhibit ovulation completely in cycling rats, while 500 μg (135 μg/kg) were required to inhibit mating-induced ovulation in rabbits (Table 4). Rabbits which ovulated in spite of antagonist treatment showed delayed and diminished LH surges. These results suggest that female rabbits are less sensitive than female rats to the inhibitory action of A_1.

Pituitary release of LH, as measured by plasma LH levels following A_1 treatment (100 μg/kg) in ovariectomized rabbits, rats and mice also suggested lower sensitivity of the rabbit and mouse pituitary (Figs 7a, b, c). Plasma LH levels in mice and rabbits reached their lowest level by 2 h and began to increase 4 h after A_1 administration, while they were still depressed in rats at 6 h. It is noteworthy that the plasma FSH of rabbits and mice was even less responsive to the inhibitory action of A_1 than LH. Plasma FSH levels of rats were also significantly less reduced than plasma LH levels. In rats A_1 was also effective at a lower dose (10 μg/kg). The differences between rats, rabbits and mice may be due to differences in receptor binding. The affinity of A_1 to pituitary receptors is 4- to 5-fold higher in rats than in rabbits [30].

Adverse effects of GnRH antagonists

Synthetic GnRH analogs are rapidly inactivated by the same enzyme systems that metabolize GnRH and are generally considered safe. During a subchronic toxicology study in rats and rabbits, standard histopathological evaluation, hematology and clinical chemistry parameters showed no evidence of A_1-induced toxic effects (Sundaram, unpublished observations). However, rats responded with acute edematous swelling of the extremities and cyanosis of the tail following subcutaneous administration of A_1 [31]. At similar doses no grossly observable effects were seen in rabbits, mice or monkeys.

The mechanisms by which A_1 produces the effects described above are not known. They do not appear to be related to GnRH antagonist activity, since two other antagonists (A_2 and A_3) had no effects in any of the species studied.

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