CONTRACEPTION AND INHIBITION OF OVULATION BY MINIPUMP INFUSION OF THE LUTEINIZING HORMONE RELEASING HORMONE, ACTIVE ANALOGS AND ANTAGONISTS*

by

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SUMMARY

[D-Phe^2,Pro^3,D-Trp^6]-LHRH, was infused subcutaneously in rats at the rate of 375 μg/day/4days by a minipump. When this antagonist was administered to cycling rats, ovulation was inhibited for no ova were found, and when administered to castrated male rats, serum LH levels were decreased. [D-Phe^2,Leu^3, D-Trp^6]-LHRH was less effective in inhibiting ovulation but also lowered the serum LH levels of castrated rats. Infusion by the minipump of 375 μg/day/4 days of LHRH and of ca. 6 μg/day/4 days of the "superactive" LHRH analog, [D-Ala^6,des-Gly^{10}]-LHRH ethylamide completely blocked the uterine implantation sites of mated rats; the two LHRH antagonists were ineffective. Activities of LHRH or its analogs responsible for post-coital contraception and prevention of ovulation are different.

The Alza Corporation, Palo Alto, California, has developed a controlled delivery device, known as a "minipump", which can be easily and rapidly inserted subcutaneously into rats and mice. The energy of this pump is based upon an osmotic driving agent in the device which is surrounded by a rate-controlling semi-permeable membrane. It allows a rate of delivery of a solution of a peptide hormone in the order of 1-2 μl/hr, during three to seven days depending upon the delivery rate and the total volume of the solution. Through the courtesy of Dr. Russell E. Phares, Jr., and Mr. Seymour Hoff, we have now used these minipumps and demonstrated the inhibition of release of LH and the inhibition of ovulation in rats by certain new and effective analogs of LHRH which are antagonists.

Humphries et al. cooperatively with Bowers (1) have reported an analog of LHRH which is a very potent inhibitor of the release of LH in vitro, and is an inhibitor of ovulation in rats by commonly used in vivo procedures of subcutaneous injection. The most potent and effective inhibitor they descibed is [D-Phe^2,Pro^3,D-Trp^6]-LHRH which was inhibitory in vitro at 50:1 for the

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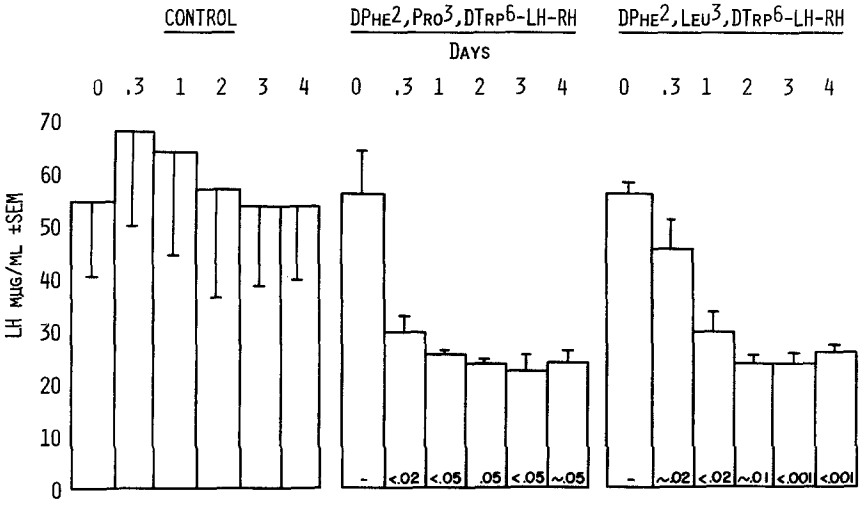

Δ Values = 0 time minus various times. p value = Δ Value of control versus experimental at respective time intervals.

Fig. 1. Effect of LH-RH analogs infused over a 4 day period from a subcutaneous Minipump on serum LH levels of castrated male rats. (Minipump was kindly supplied by Alza Corp., Palo Alto, Calif.)

ratio of analog:LHRH. This antagonist caused complete inhibition of ovulation after a single subcutaneous injection into rats of dosages of 750 μg, and 50% inhibition of ovulation at 375 μg.

The data in Chart I show the diminution of levels of LH when the two related analogs were administered subcutaneously to castrated male rats by the minipump over a period of 4 days. Control levels of LH in the serum ranged from about 55 to 68 ng/ml over the 4-day period, when 50% propylene glycol was used as the vehicle.

The continuous delivery over 4 days by a minipump at 375 μg/day of [D-Phe^2,Pro^3,D-Trp^6]-LHRH showed that the levels of LH decreased to a range of about 20 to 30 ng over the 4 day period, $P < 0.05$. The effect of [D-Phe^2, Leu^3,D-Trp^6]-LHRH was similar.

The data in Table I show that the number of ova per ovulating rat was 15.4 ± 1.8 when the minipump was used with propylene glycol as the control vehicle. When [D-Phe^2,Pro^3,D-Trp^6]-LHRH was subcutaneously delivered by the minipump at the rate of 375 μg per day for 4 days, there were no ova and the inhibition of ovulation was complete.

The corresponding [D-Phe^2,Leu^3,D-Trp^6]-LHRH, delivered under the same

TABLE I. EFFECT OF LHRH ANALOGS CONSTANTLY INFUSED OVER A 4 DAY PERIOD FROM A SUBCUTANEOUS MINIPUMP ON OVULATION OF RATS

Analog	Dose mg/rat s.c.	No. of rats	No. of rats ovulated	No. of ova/ ovulating rat ± SEM	% inhibition of ovulation
50% PG *	--	5	5	15.4±1.8	0
[D-Phe^2,Pro^3,D-Trp^6]-LHRH	1.5 mg	4	0	0±0	100
[D-Phe^2,Leu^3,D-Trp^6]-LHRH	1.5 mg	5	4	10.8±2.7	20

*Propylene glycol

TABLE II. DATA ON UTERINE IMPLANTATION SITES OF PREGNANT RATS AFTER INFUSION BY THE MINIPUMP

Peptide *	Dose µg/rat	No. of rats	Implantation Sites normal/total	Corpus Luteum
Control	---	4	13.0±0.9/13.0±0.9	14.0±1.2
LHRH	1500	4	0±0 / 0±0	21.0±1.5
Control	---	6	11.8±0.5/11.8±0.5	13.7±0.7
LHRH	500	5	0±0 / 5.0±3.1	13.3±4.5
[D-Ala^6,des-Gly^{10}]-LHRH-EA	25	6	0±0 / 0±0	25.6±0.8
Control #2 (PG)	---	5	12.2±0.6/12.2±0.6	15.5±0.5
[D-Ala^6,des-Gly^{10}]-LHRH-EA (PG)	25	5	0±0 / 0±0	30.0±0.5
[D-Phe^2,Pro^3,D-Trp^6]-LHRH (PG)	1500	5	13.0±0.8/13.0±0.8	14.6±1.3
[D-Phe^2,Leu^3,D-Trp^6]-LHRH (PG)	1500	5	12.2±0.7/12.2±0.7	14.1±1.0

* EA = ethylamide
PG = propylene glycol

conditions at the same dosage, resulted in 10.8 ± 2.7 ova per ovulating rat which corresponds to an inhibition of ovulation of about 20%.

Corbin and Beattie (2) found that complete inhibition of uterine implantation sites (pregnancy) in rats was demonstrated when a total dose of 7 mg LHRH/rat was subcutaneously administered at a daily dose of 1 mg over days 1-7. LHRH was also effective as an "interceptive", since pregnancy was terminated in all rats when it was administered from days 7-12 of pregnancy at a daily dose of 1 mg/rat, sc or p.o. Similar results were obtained in rabbits. Also, it was found that LHRH effectively prevents conception when administered before ovulation. Lin and Yoshinaya (3) showed that LHRH had a post-coital contraceptive effect.

Our data, in Table II, show that when synthetic LHRH was infused by the minipump at a daily level of 375 µg/rat/day/4 days there were no uterine implan-

tation sites, and at 125 μg, there was partial implantation. The minipump was inserted sc on the a.m. of estrus after the presence of sperm was found in the vaginal secretion and the corpus luteum and uterine implantation sites were counted under a dissection microscope on day 8 of gestation. There were no implantation sites when the "superactive" LHRH analog, [D-Ala^6,des-Gly^{10}]-LHRH ethylamide, was infused by the minipump at a daily dosage of only 6 μg/rat/day/ 4 days. These results from the minipump confirm, in principle, the data of Corbin and Beattie (2) and underscore the potentiality of LHRH to be an important contraceptive agent as well as an agent to increase fertility.

The data in Table II show that [D-Phe^2,Pro^3,D-Trp^6]-LHRH and [D-Phe^2, Leu^3,D-Trp^6]-LHRH at the same dosage using the minipump did not block implantation after mating. The mechanisms of action of LHRH and "superactive" analogs of LHRH which cause post-coital contraception, and the mechanisms of antagonists of LHRH which prevent ovulation are seemingly different. It was considered (2) that contraception might be based on hyperstimulation of the hypophysial-ovarian steriod-uterine axis or on a direct extrapituitary (uterine) effect or perhaps on the suppression of estradiol and progesterone secretion despite continued elevated serum LH levels (3).

Although serum LH and FSH levels were not measured during the first 4 days of pregnancy while infusing 375 μg/day of LHRH by the minipump, the same amount and method of delivering LHRH to normal adult male rats (unpublished) markedly elevated serum LH (70-100 fold) and FSH (ca. 7 fold) levels on the first day of the infusion but, thereafter, LH levels only rose 2 to 4 fold on days 2 and 3 and none on day 4; FSH failed to rise after day 1. If these same LH and FSH changes occured in pregnant rats when the same amount of LHRH was infused by the minipump for 4 days, it could indicate that the LHRH post-coital contraception effect observed in our studies is mediated by a different mechanism than those observed by Lin and Yoshinago (3). Undoubtedly, the mechanism of the LHRH post-coital contraceptive effect could be complex and multiple being in part dependent on the dose and duration of administration of LHRH or an LHRH analog with agonist activity. Similarly, the mechanisms of ovulation inhibition by LHRH analog antagonists may not always be by a direct competitive inhibition with LHRH on the pituitary, for it has been found that the inhibition, *in vitro*, of the release of LH and FSH by LHRH and the antiovulatory activity of these analogs do not always parallel each other (4).

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