PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 21–24 (1979)

Inhibition of Gonadotropin Release in Chimpanzees by the LH-RH Antagonist (D-Phe^2, D-Trp^3, D-Phe^6)-LH-RH (40480)

ROBERT E. GOSSELIN, GENE B. FULLER, DAVID H. COY, ANDREW V. SCHALLY, AND WILLIAM C. HOBSON

International Center for Environmental Safety, Albany Medical College, Holloman Air Force Base, New Mexico 88330, and, Veterans Administration Hospital and Tulane University School of Medicine, New Orleans, Louisiana 70146

The chimpanzee (*Pan troglodytes*) is an excellent model for gonadotropin studies since its pattern of gonadotropin release is similar to that of humans (1–3). Chimpanzees also respond to stimulation by LH-RH in a manner closely approximating the human response (4), whereas the response of other non-human primate species is inconsistent (4–8).

Several hundred analogs of LH-RH have been synthesized in recent years. In rats, both ovulation and the release of gonadotropins following LH-RH injection can be blocked by prior administration of antagonistic LH-RH analogs (9). The extent of inhibition of LH-RH activity is dependent on the structure of the analog. Substitution of the amino acids at the 2, 3, and 6 positions of the decapeptide has produced antagonists with the greatest inhibitory properties to date (9). One of the most potent of the antagonists, (D-Phe^2, D-Trp^3, D-Phe^6)-LH-RH has been shown to inhibit the responses to LH-RH in humans (10, 11). In this communication, we report on the ability of this analog to block the effects of exogenous LH-RH in the chimpanzee.

Materials and methods. Animals. The chimpanzees used in the study were caged in pairs and fed a diet of monkey biscuits and fresh fruit. A total of 12 females ranging in age from 8 to 21 years and weighing from 34 to 60 kg were utilized. Daily records of sex-skin swelling and menstruation were maintained. The animals selected for the study were in the early periovulatory phase of the menstrual cycle, a period which corresponds to Days 5–7 of maximal sex-skin swelling.

Procedure. In order to reduce variability in endogenous gonadotropin release all experiments were begun at 8:00 AM. The animals were anesthetized with an initial dose of Ketamine HCl (10 mg/kg, im). Anesthesia was maintained by repeated 5 mg/kg doses as needed for the duration of the experiment. The response to LH-RH has been shown to be similar in conscious and anesthetized chimpanzees (4).

The animals were divided into 3 groups of 4 chimpanzees each. The LH-RH analog (Coy, Schally) was dissolved in 5 ml of propylene glycol-saline, 3:2, v/v. At time 0, the control, low dose and high dose groups were injected with vehicle, 7 or 35 mg of analog respectively. LH-RH (10 μg in 1 ml saline, iv) was given to all animals at 30 and 120 min following administration of the antagonist or vehicle. Blood samples were collected from the femoral vein at -10, -5, 0, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 130, 140, 150, 180, and 210 min from the time of the antagonist or vehicle injection. Samples were allowed a minimum of 1 hr to clot and the sera were separated by centrifugation and stored frozen.

Assays. Radioimmunoassays for chimpanzee FSH and LH have been previously validated (12) and were performed as described by Howland *et al.* (12). The human pituitary preparation LER 907 (20 IU FSH, 48 IU LH 2nd IRP/mg) was used as a standard for both chimpanzee LH and FSH. Chimpanzee gonadotropins cross react with antibodies to human gonadotropins in such a manner that dilutions of chimpanzee sera are parallel to the human pituitary standard (12). Assay reliability was assessed by the quality control system of Rodbard *et al.* (13). All values represent the mean of duplicate determinations.

Results. The LH response of a representative animal from each group is presented in Fig. 1. The three animals shown had similar endogenous levels of serum LH prior to injection of the analog.

0037-9727/79/050021-04$01.00/0

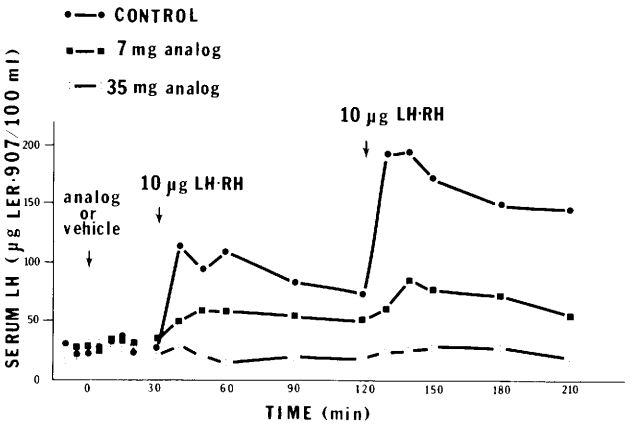

FIG. 1. LH release in an individual representative animal from each of the control and analog treated groups. At time 0, the animal from the low dose group was injected (S.C.) with 7 mg of the LH-RH analog in 5 ml of propylene glycol–saline, 3:2, v/v, while the high dose animal received 35 mg of the analog in the same amount of vehicle and the animal from the control group was injected with the vehicle only. At 30 and 120 min, each animal received 10 μg of LH-RH in 1 ml of saline, iv.

The experiment was divided into four segments based on the times of analog and LH-RH injections. The experimental segments were labeled Baseline, Analog, First LH-RH and Second LH-RH (See Fig. 2 legend for sample times in each segment). Values for each treatment group were pooled within each experimental segment and compared with the pooled values in the corresponding experimental segment of the control group by analysis of variance and the least-significant-difference test. Each group was comprised of four animals, however, one of the control chimpanzees became pregnant just prior to the time of the experiment and data from this animal were not used. The LH and FSH values for each animal were expressed as a percent of its own preinjection baseline levels in order to reduce any variation due to different basal gonadotropin levels. Endogenous levels of both LH and FSH for all the animals studied were not affected in the 30 min between analog or vehicle administration and the first LH-RH dose (Figs. 1 and 2). Mean LH concentrations in the control animals rose more than 100% over baseline following the first dose of LH-RH. The analog, at the 35 mg dose, significantly ($p < 0.05$) reduced the LH response to the first LH-RH injection. The response to the second dose of LH-RH was inhibited even more. In this case, the group treated with the 35 mg dose showed a highly significant reduction from controls ($p < 0.01$) indicating that the inhibition of LH release lasts for at least 3 hr following injection of the analog. The responses of the group treated with the 7 mg dose showed a similar pattern of inhibition, but the difference from controls was much smaller than with the 35 mg group and was not statistically significant.

The high variability of the FSH responses to LH-RH in some animals precluded a definitive interpretation of the effects of the analog on FSH release.

Discussion. These experiments demonstrated the ability of a synthetic LH-RH antagonist to suppress LH-RH-induced gonadotropin release in a primate species which is phylogenetically very close to man. The inconsistent responses of other non-human primate species to LH-RH have made previous studies in this area difficult to interpret. In order to rapidly develop, test and establish the safety of LH-RH analogs with contraceptive potential an appropriate non-human primate model of man is necessary.

The dose of LH-RH (10 μg) was selected from preliminary studies which showed that this was the lowest amount which would consistently release LH in periovulatory chimpanzees. The magnitude of LH release in response to the LH-RH injections was reduced by the 35 mg dose of the antagonistic analog to approximately 33% of the amount released in the control animals. The smaller reductions caused by the 7 mg dose were not

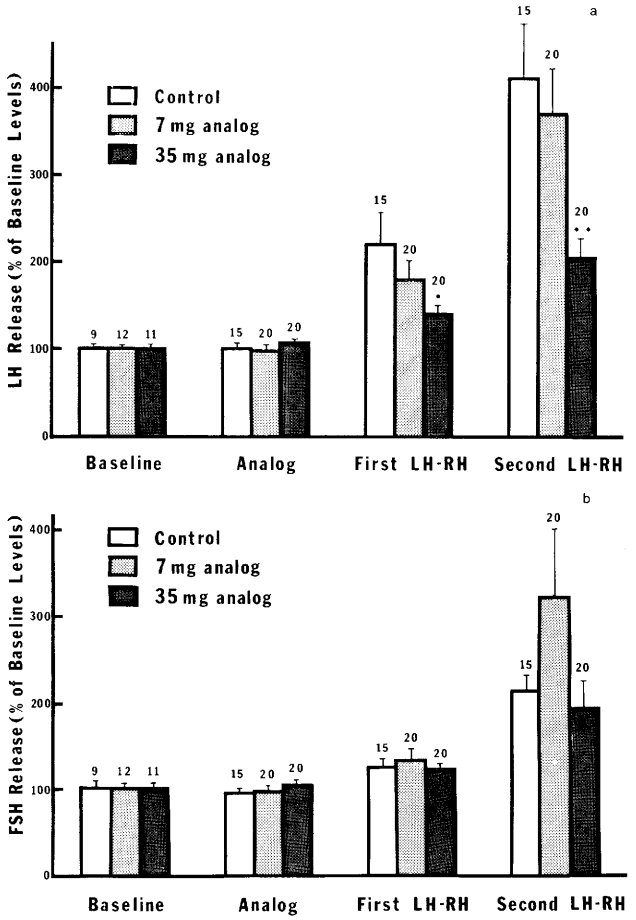

FIG. 2a and b. LH and FSH release in chimpanzees treated with LH-RH analog or vehicle followed by LH-RH. Values are expressed as a percent of the mean of pretreatment baseline values. The bars represent the means of pooled data for each group of animals from the following time periods; Baseline (−10, −5, 0 min), Analog Injection (5, 10, 15, 20, 30 min), First LH-RH Injection (40, 50, 60, 90, 120 min), Second LH-RH Injection (130, 140, 150, 180, 210 min). The numbers above the bars indicate the number of observations for that group. Vertical lines represent the standard error of the mean. *, $p < 0.05$;**, $p < 0.01$.

significant. These data suggest that the minimum effective dose was between 7 and 35 mg and that the effects of the analog persist for at least 3 hr. This work is in agreement with similar studies conducted in humans in which 90 mg of the same analog inhibited the response to exogenous LH-RH administration in normal men for as long as 24 hr and lowered the high levels of LH and FSH in postmenopausal or ovariectomized women (12, 13).

It was interesting to find that while the analog inhibited the response to exogenous LH-RH, basal levels of LH and FSH, regardless of magnitude, were not immediately affected in the 30 min between analog injection and the first LH-RH challenge. The value of LH-RH analogs as contraceptive agents would be enhanced by subsequent demonstration that they block the midcycle gonadotropin surge but do not affect basal levels of circulating LH and FSH. Of equal importance is the absence of enduring effects of the analog which would disrupt normal menstrual cycling. The analog used in the current study had no apparent lasting effects since all the animals which were treated continued to cycle normally and those which were subsequently mated showed positive pregnancy results.

The biological potency of the analogs, and eventually their ability to inhibit ovulation will probably depend to a large extent on their resistance to degradation thereby ensuring retention of their *in vivo* activity for extended periods of time. While both the potency and biologically-effective life of several analogs have been investigated in rats (9), these parameters have not been examined in primates. The availability of supporting data from primates would enhance the usefulness of the rat model in predicting the relative effectiveness of these potential contraceptive agents in man.

Summary. Thirty-five mg of the LH-RH antagonist (D-Phe^2, D-Trp^3, D-Phe^6)-LH-RH inhibited the LH response of chimpanzees given 10 μg of LH-RH to 33% of the amount released by control animals. Seven mg of the same analog also attenuated the LH response, but the inhibition was not statistically significant. Neither dose level of the inhibitor significantly altered basal gonadotropin levels. These data indicate that LH-RH antagonists have at least an acute ability to diminish the response to exogenous releasing hormones without acutely affecting basal gonadotropin secretion. They further suggest that a non-human primate model may be used to test the efficacy of these compounds as potential contraceptive agents.

The authors wish to acknowledge The National Institutes of Health, Center for Population Research for the gift of LH-RH and The National Institute of Arthritis, Metabolism, and Digestive Diseases for the gifts of LER-907 Standard and their antiserum #4 against human FSH used in radioimmunoassay. We are grateful to Dr. C. Faiman, University of Manitoba, who supplied the antibodies used in the LH assay. We also thank Dr. Robert Veenstra, Mr. Joseph Murray and Mr. Paul Gonzales for their assistance with the animals and Ms. Becky Sims for manuscript preparation.

This work was supported by the Contraceptive Development Branch, Center for Population Research, NICHHD, National Institute of Health, Bethesda, MD. We are grateful to Dr. Marvin Karten for helpful advice and encouragement.

1 258 1. Hobson, W. C., Coulston, F., Faiman, C., Winter, J. S. D., and Reyes, F. I., J. Tox. Environ. Health **1,** 657 (1976).
2. Winter, J. S. D., Faiman, C., Hobson, W. C., Prasad, A. V., and Reyes, F. I., J. Clin. Endocrinol. Metabol. **40,** 545 (1975).
3. Reyes, F. I., Winter, J. S. D., Faiman, C., and Hobson, W. C., Endocrinology **96,** 1447 (1975).
4. Hobson, W. C., and Fuller, G. B., Biol. Reprod. **17,** 294 (1977).
5. Ehara, Y., Ryan, K. S., and Yen, S. S. C., Contraception 6, 465 (1972).
6. Ferin, M., Warren, M., Dyrenfurth, I., Van De Wiele, R. L., and White, W. F., J. Clin. Endocrinol. Metabol. **38,** 231 (1974).
7. Shah, G. V., Sheth, A. R., Godgil, B. A., and Swamy, X. R., Indian J. Exp. Biol. **14,** 171 (1976).
8. Mori, J., and Hafez, E. S. E., J. Reprod. Fert. **34,** 155 (1973).
9. Schally, A. V., Coy, D. H., and Myers, C. A., Ann. Rev. Biochem. **45,** 89 (1978).
10. Gonzalez-Barcena, D., Kastin, A. J., Schally, A. V., Coy, D. H., Vilchez-Martinez, J. A., Pedroza-Garcia, E., Nikolics, K., and Seprodi, J., Fert. Steril. **29,** 246 (abstract) (1978).
11. Gonzalez-Barcena, D., Kastin, A. J., Coy, D. H., Nikolics, K., and Schally, A. V., Lancet **2,** 997 (1977).
12. Howland, B. E., Faiman, C., and Butler, T. M., Biol. Reprod. **4,** 101 (1971).
13. Rodbard, D., Rayford, P. L., Cooper, J. A., and Ross, G. T., J. Clin. Endocrinol. Metabol. **28,** 1412 (1968).

Received October 20, 1978. P.S.E.B.M. 1979, Vol. 161.