2-HYDROXYESTRONE INDUCED RISE IN SERUM LUTEINIZING HORMONE IN THE IMMATURE MALE RAT

by

F. Naftolin, H. Morishita, I.J. Davies,
R. Todd and K.J. Ryan

Harvard Medical School
Department of Obstetrics and Gynecology
Laboratory of Human Reproduction and Reproductive Biology
45 Shattuck Street
Boston, Massachusetts 02115

and

J. Fishman

Institute of Steroid Research
Montefiore Hospital and Medical Center
111 East 210th Street
Bronx, New York 10467

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SUMMARY

Subcutaneous injection of 50 or 100 µg of 2-hydroxyestrone in 35 day old male rats resulted in a dramatic (4-7 fold) rise in serum luteinizing hormone at 6 hours post treatment. Estrone in similar doses (1-200 µg) caused a small decrease in serum luteinizing hormone which was not statistically significant. The discrepancy between the actions of naturally occurring estrogens may be useful in explaining the time and dose related biphasic effects of estrogens on gonadotropin levels.

The action of estrogens in controlling gonadotropins is well documented and has had universal clinical application in manipulation of fertility (1). Estrogens have effects which are both time and dose related. They can cause elevations and/or suppression of circulating gonadotropins under different conditions of age, sex, gonadal status, species, etc. (2-6). While these apparently inconsistent effects of estrogen have been extensively described, little is known of the underlying mechanisms involved or of the role of the products of estrogen metabolism in estrogen action.

The recent proof of *in situ* formation of estrogens (7) and

their 2-hydroxylated metabolites (8) in central tissues of rodents has led us to systematically explore the effects of these compounds on central reproductive mechanisms. In a series of experiments we have administered estrone (E_1) and 2-hydroxyestrone (2-OHE_1) to immature male rats to assess their effects upon circulating gonadotropin levels.

METHODS: Male Sprague-Dawley derived rats were purchased from Charles River Breeding Laboratories at 28 days of age and injected subcutaneously with vehicle alone (propylene glycol with 0.01% ascorbic acid) or test steroid in vehicle on day 35 of life. After 6 hours the animals were anesthetized by inhalation of a 50% O_2:CO_2 mixture and 2-3 ml of blood was aspirated via cardiac puncture. Animals had been distributed so that there was no significant weight difference between experimental groups ($p>0.25$). Samples were handled and assayed for serum luteinizing hormone (LH) as previously reported (9). The error of the method is <5% of intra-assay variation and all samples from each experiment were run in the same assay. 2-OHE_1 was prepared by the method of Fishman, et al. (10). It was purified and proved homogenous by paper and thin layer chromatography followed by intentional contamination with ^{3}H-estradiol, acetylation and recrystallization of the di-acetate to complete absence of radioactivity. The di-acetate was then hydrolyzed to furnish pure 2-OHE_1.

RESULTS: Control levels for LH in these experiments were the same as we have reported previously (9), and also were the same for each of the 3 groups of 10 animals used for controls in the present study ($p>0.25$).

Estrone generally lowered the mean LH levels in these animals; however, difference from the controls was not statistically significant (paired "t" test). There was little potency difference between doses of 1-200 µg of E_1 in terms of the minimum LH level reached, but the effect was more regular at the highest dose. In 1 and 10 µg doses, 2-OHE_1 had no clear effect on LH levels by 6 hours after injection. However, in all three experiments, 2-OHE_1 in doses of 50 and 100 µg elevated serum LH by 4-7 fold ($p<0.05$ to <0.001). In the dose response experiment, the elevation appeared to peak at the 50 µg dose and diminish

with increasing doses (i.e., the p for experimental versus control was <0.01 for 50 µg, <0.05 for 100 µg and >0.05 for 200 µg).

DISCUSSION: Our findings in these preliminary studies are of interest on several counts. While intraventricular catecholamine administration is very active in elevating circulating gonadotropin levels in rats, parenterally administered catecholamines appear to have little or no effect (11-12). In contrast, parenteral administration of the catechol estrogen, 2-OHE_1, causes a rapid and definite elevation of LH. Thus, the transport of these two classes of compounds into the central axis may be different. In any case, since in vitro production of catechol estrogens in central neuroendocrine tissues from rats (8) and humans (unpublished) has been demonstrated, the necessity

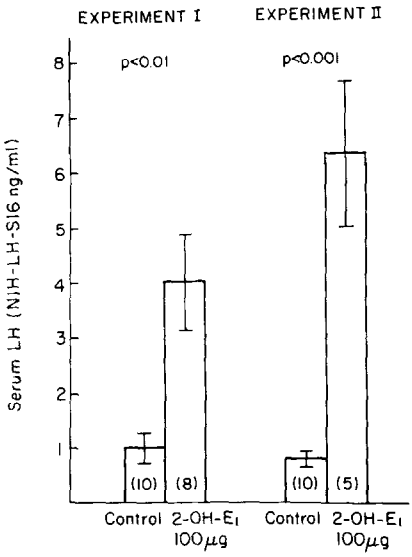

Figure 1. Experiments I and II:
Serum LH (mean ± S.E.) in 35 day old male rats 6 hours after injection of vehicle or 2-OHE_1. The number of animals is in parentheses. P values are for control vs. experimental in each experiment.

for endogenous catechol estrogen to enter central tissues is not established.

Perhaps of greatest interest is the elevation of LH levels by 2-OHE_1, especially since the parent estrogen appears to effect no change or to lower LH in these animals. Furthermore, the clear elevation of gonadotropin in male rats is noteworthy since the male rat lacks biphasic feedback in all models thus far tested (13,14). It is possible that 2-OHE_1 acts via competition for cytosol binding sites in the same manner as the non-steroidal antiestrogen clomiphene which has been used to induce ovulation in women (1). This idea is consistent with the peaked dose response curve, the rapidity of action, and the recent finding that 2-OHE_1, although a poorly vaginotropic hormone (15), actively competes with ^3H-estradiol binding to estrogen receptors in hypothalamic and pituitary cytosol in both humans (16) and rats (I.J. Davies, et al., submitted for publication). Of

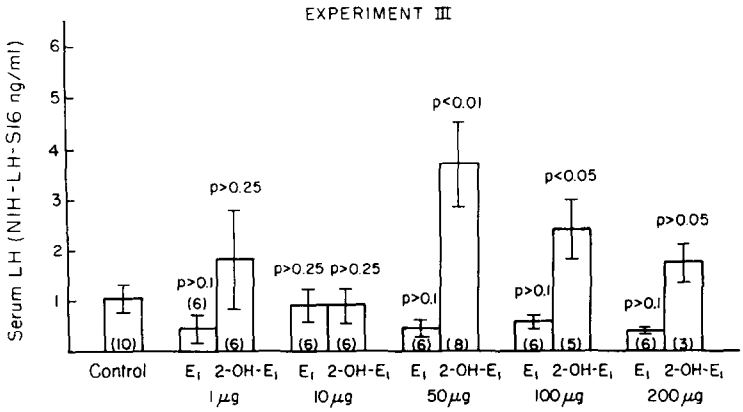

Figure 2. Experiment III:
Serum LH (mean ± S.E.) in 35 day old male rats 6 hours after injected vehicle, E_1 or 2-OHE_1 in doses noted beneath the abscissa. P values are for control vs. experimental.

course, an effect upon brain active amine metabolism may also be present (17-27). Whatever the case, it is clear the 2-OHE_1 can rapidly elevate LH in male rats, the magnitude of the effect approaching that of the ovulatory surge of LH in females (28).

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REFERENCES:

1. Harris, G.W., and Naftolin, F. (1970) Br. Med. Bull., 26, 3.
2. Goding, J.R., Catt, K.J., Brown, J.M., Kaltenback, I.A., and Mole, B.J. (1969) Endocrinology, 85, 133.
3. Tsai, C.C., and Yen, S.S.C. (1971) Endocrinology, 33, 917.
4. Monroe, S.E., Jaffe, R.B., and Midgley, A.R. (1972) J. Clin. Endocrinol., 34, 342.
5. Karsch, F.J., Dierschke, D.J., and Knobil, E. (1973) Science, 179, 484.
6. Dierschke, D.J., Weiss, G., and Knobil, E. (1974) Endocrinology, 94, 198.
7. Naftolin, F., Ryan, K.J., Davies, I.J., Reddy, V.V., Flores, F., Petro, Z., and Kuhn, M. (1975) Recent Progr. Horm. Res., 31, (In press).
8. Fishman, J., and Norton, B. Endocrinology (In press).
9. Naftolin, F., and Feder, H.H. (1973) J. Endocrinol., 56, 155.
10. Fishman, J., Tomasz, M., and Lehman, R. (1960) J. Organic Chem., 25, 585.
11. Schneider, H.P.G., and McCann, S.M. (1970) J. Endocrinol., 46, 401.
12. Kamberi, I.A., Mical, R.S., and Porter, J.C. (1971) Endocrinology, 88, 1003.
13. Harris, G.W. (1964) Endocrinology, 75, 627.
14. Neill, J.D. (1972) Endocrinology, 90, 1154.
15. Martin, L. (1965) In Estrogen Assays in Clinical Medicine, Paulsen, A., University of Washington Press, Seattle, 125-128.
16. Davies, I.J., Naftolin, F., Ryan, K.J., and Siu, J.,J. Clin. Endocrinol. Metab. (In press).
17. Vogel, S.A., Janowsky, D.J., and Davis, S.M. (1970) Res. Comm. Chem. Path. Pharmacol., 1, 451.
18. Cohn, C.K., and Axelrod, J. (1971) Life Sci., 10, 1351.
19. Raziano, J., Cowchock, S., Ferin, M., and Vande Wiele, R. (1971) Endocrinology, 88, 1516.
20. Klaiber, E.L., Kobayashi, Y., Broverman, D.M., and Hall, F. (1971) J. Clin. Endocrinol., 33, 630.
21. Shlemus, S., Engel, J., Eriksson, H., Modigh, K., and Sodersten, J. (1972) Neural Trans., 33, 247.
22. Ball, P., Knuppen, R., Haupt, M., and Breuer, H. (1972) J. Clin. Endocrinol., 34, 736.
23. Beattie, C.W., Rodgers, C.H., and Soyka, L.F. (1972) Endocrinology, 91, 276.

24. Gunaga, K.P., and Menon, K.M.J. (1973) Biochem. Biophys. Res. Comm., 54, 440.
25. Parvez, H., and Parvez, S. (1973) J. Neurochem., 20, 1011.
26. Simpson, C.W., and Dicara, L.V. (1973) Pharmacol. Biochem. Behav., 1, 413.
27. Stern, J.J., and Zwick, G. (1973) Behav. Biol., 9, 605.
28. Naftolin, F., Brown-Grant, K., and Corker, C.S. (1972) J. Endocrinol., 53, 17.