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Effect of a Long-Acting Estrogen on the Pituitary Response to LH-Releasing Hormone (38582)

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(Introduced by A. L. Gimeno)

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In a series of reports it was demonstrated that estradiol augmented the pituitary response to LH-releasing hormone (LH-RH) in female and castrated male rats (1–3). Since estradiol was able to modify the release of LH by pituitaries when added to an incubation medium *in vitro* (4) or when implanted *in vivo* into the pituitary gland (5), it seems evident that this effect of estradiol can be exerted, at least in part, directly on the pituitary gland (6). Moreover, it has been recently demonstrated that the effect of estradiol on the pituitary response to LH-RH in rats was biphasic, an early effect being inhibitory, and a later one being stimulatory (7). In the present investigation it was decided to study the effect of an estrogen with long-lasting activity on the pituitary response to LH-RH at different time periods after the administration of the steroid. With this aim in mind, a long-acting estrogen, Quinestrol,[2] was selected for the study. Quinestrol was previously demonstrated to be an estrogen with long-lasting activity due to its accumulation and slow release from the adipose tissue (8).

Materials and Methods. Adult chronically ovariectomized rats of the Wistar strain were used. The animals were kept in quarters equipped with controlled temperature and illumination (14 hr light–10 hr darkness) and were fed Purina laboratory chow, fresh vegetables, and water *ad lib.* The rats were divided into three groups of 14 rats each. The animals of the first group were given 1 ml of 1% tragacanth gum by oral route and were considered as controls. The rats of the second group were treated with 20 μg of Quinestrol per rat, given by oral route as a suspension in 1% tragacanth gum, and the rats of the third group were similarly treated, but each rat received 200 μg of Quinestrol. In all the cases Quinestrol was administered as a single dose. At variable intervals after the treatment, namely 36 hr, 9 days, 24 days, and 36 days, the rats of each group were injected iv under light ether anesthesia either with acidified saline (7 rats) or with acidified saline containing 20 ng of synthetic LH-RH/rat (7 rats). Twenty minutes later blood was drawn with a syringe through the jugular vein. Sera were separated by centrifugation and kept frozen. LH was assayed in each serum sample using the double-antibody radioimmunoassay described by Niswender *et al.* (9). Ovine LH was iodinated with ^{125}I and NIAMDD-Rat LH-RP1 was used as a standard preparation. The significance of the differences among means was investigated applying the Tukey's test to the log of the data (10).

Results. The results will be considered separately in each of the time intervals after treatment. (a) Thirty six hours after the administration of the vehicle alone the control rats injected with saline showed elevated serum LH levels as compared with previously reported serum LH levels in normal rats using the same assay method in our laboratory (11) (Fig. 1). In LH-RH-injected rats serum LH levels were not significantly different from those of saline-injected rats. In the group treated with 20 μg of Quinestrol, serum LH levels in saline-injected rats were lower than the respective LH levels in control rats although the difference was not significant. The rats injected with LH-RH within the same group showed serum LH levels significantly higher than those of saline-

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[2] Quinestrol is the trivial name of 3-cyclopentyl ether of ethinyl estradiol.

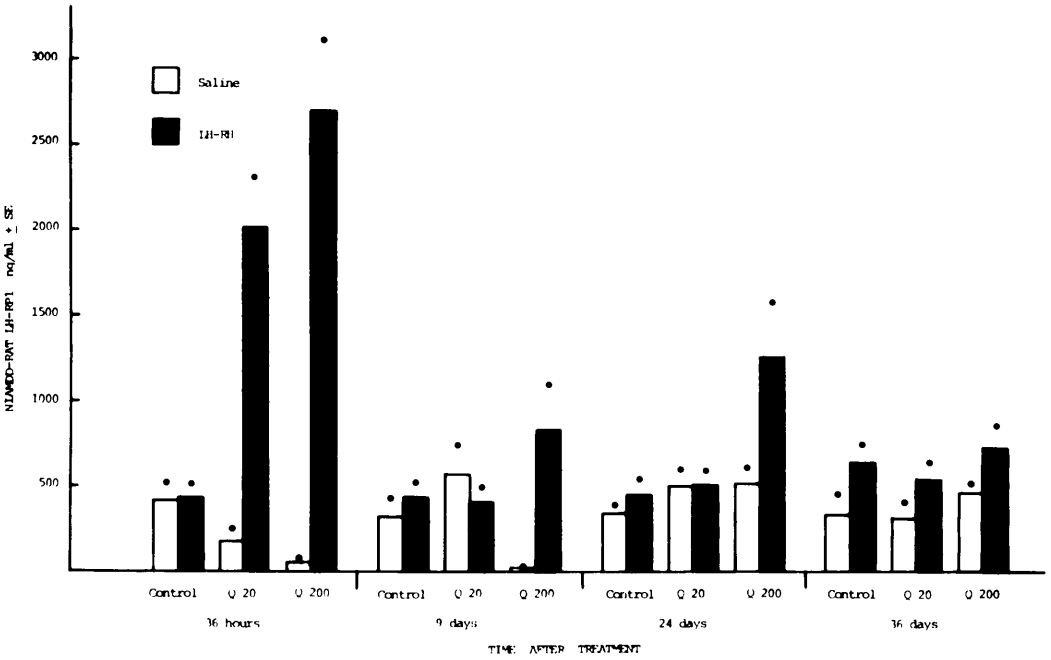

FIG. 1. Effect of two doses of Quinestrol on the pituitary response to LH-RH at different time periods. The results are expressed as ng/ml of NIAMDD-Rat LH-RP1 ± SE. Q 20: Quinestrol 20 μg; Q 200: Quinestrol 200 μg.

injected rats ($P < 0.01$). In the group treated with 200 μg of Quinestrol, serum LH levels from saline-injected rats were significantly lower than the respective values in the previous groups ($P < 0.01$). Serum LH levels in LH-RH-injected rats were significantly higher than those of saline-injected rats ($P < 0.01$). (b) Nine days after treatment in the control group as well as in the group treated with 20 μg of Quinestrol, saline-injected rats showed no significantly different serum LH levels. No significant differences were seen in LH-RH-injected rats as compared with saline-injected rats in their respective group. The saline-injected rats from the group treated with 200 μg of Quinestrol had serum LH levels significantly lower than the saline-injected rats from the other two groups ($P < 0.01$). Serum LH in LH-RH-injected rats treated with 200 μg of Quinestrol was significantly higher than the corresponding serum LH levels in saline-injected rats ($P < 0.01$). (c) Twenty four days after treatment serum LH levels in control rats as well as those in rats treated with 20 μg of Quinestrol were within similar limits in the respective saline or LH-RH-injected rats. Serum LH from saline-injected rats treated with 200 μg of Quinestrol showed no significant differences as compared with those of the other two groups, and serum LH levels from LH-RH-injected rats treated with 200 μg of Quinestrol were not significantly different either, in spite of the fact that they were considerably higher than the serum LH levels in saline-injected rats within the same group. This apparent lack of significance could be due to the relative insensitivity of the statistical test used. (d) Thirty six days after treatment, serum LH levels from saline-injected rats were similar in the three different groups, control and Quinestrol-treated. Serum LH levels from control and Quinestrol-treated rats injected with LH-RH were not significantly different from their respective saline-injected rats, in spite of the fact that all the groups injected with LH-RH had higher serum LH values than the respective saline-injected groups.

Comparing the serum LH levels of LH-RH-injected rats from all the four periods after treatment, the highest serum LH levels were observed at 36 hr after treatment in rats given 20 and 200 μg of Quinestrol ($P <$

0.01). These two values were not significantly different from each other. The third highest LH level was observed in LH-RH-injected rats 24 days after treatment with 200 μg of Quinestrol.

Discussion. From the results presented it is evident that at the dose used LH-RH did not induce a significant LH increase in serum in any of the control groups. Quinestrol depressed the basal serum LH levels when administered in a 200 μg-dose up to at least 9 days. Twenty four days after the administration of Quinestrol the basal serum LH levels were similar to those of control rats. This effect was evidently exerted through a negative feedback as was previously demonstrated with other estrogen preparations (12). At the 20 μg-dose, Quinestrol depressed basal serum LH levels only at the 36 hr-interval studied. Quinestrol sensitized the pituitary gland to LH-RH and therefore markedly augmented the LH release after the injection of the decapeptide. The effect of Quinestrol at the dose of 20 μg, however, had a short and transient influence on the pituitary responsiveness to LH-RH, since its sensitizing effect was evident only 36 hr after its administration as a single dose. The effect of 200 μg of Quinestrol was more prolonged since basal serum LH levels 9 days after the administration of the steroid were still significantly lower than the respective serum LH levels in the other two groups. In spite of the fact that 24 days after treatment, basal serum LH levels in rats treated with 200 μg of Quinestrol were not significantly different from those of the other two groups, the pituitary response to LH-RH was still augmented. No Quinestrol effect was evident 36 days after its administration since the pituitary response to LH-RH during that time period was quite similar in the three groups. It can be concluded that 200 μg of Quinestrol administered to ovariectomized rats exert an augmenting effect on the pituitary response to LH-RH up to a period of at least 24 days. On the other hand, at a dose ten times lower, Quinestrol exerts a marked stimulatory effect on the pituitary response to LH-RH, but such effect is apparently short-lived. If the same sensitizing effect of Quinestrol could eventually be demonstrated in the human, one may speculate that a combined treatment with a single periodic adminstration of Quinestrol might be useful to stimulate the hypothalamus through a positive feedback effect, and also sensitizing the pituitary gland, thus augmenting its responsiveness to LH-RH. Theoretically this type of treatment might be useful in the management of some cases of hypothalamic amenorrheas.

Summary. The effect of Quinestrol, an estrogenic substance of long-lasting activity, on the pituitary responsiveness to LH-RH was studied in ovariectomized rats. Quinestrol administered as a single 200 μg-dose significantly augmented the pituitary response to LH-RH 36 hr, 9 days, and, although not significantly, also at 24 days after its oral administration. No effect could be detected 36 days after its administration. At a 20 μg-dose Quinestrol significantly augmented the pituitary response to LH-RH only at the 36 hr-period studied, this effect not being apparent in later time periods.

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1. Debeljuk, L., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **139,** 774 (1972).
2. Debeljuk, L., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **143,** 1164 (1973).
3. Debeljuk, L., Vílchez-Martínez, J. A., Arimura, A., and Schally, A. V., Endocrinology **94,** 1519 (1974).
4. Schally, A. V., Redding, T. W., and Arimura, A., Endocrinology **93,** 893 (1973).
5. Kingsley, T. R., and Bogdanove, E. M., Endocrinology **93,** 1398 (1973).
6. Debeljuk, L., Arimura, A., and Schally, A. V., Endocrinology **90,** 1578 (1972).
7. Vílchez-Martínez, J. A., Arimura, A., Debeljuk, L., and Schally, A. V., Endocrinology **94,** 1300 (1974).
8. Giannina, T., Steinetz, D. G., and Meli, A., Int. J. Fert. **12,** 142 (1967).
9. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128,** 807 (1968).

10. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York (1960).
11. Debeljuk, L., Rozados, R., Daskal, H., and Villegas Vélez, C., Neuroendocrinology, in press.
12. Davidson, J. M., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. F. Ganong, eds.), p. 343. Oxford University Press, London (1969).

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