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Effect of Actinomycin D and Estradiol on the Response to LH-Releasing Hormone in Neonatally Androgenized Female Rats[1] (39008)

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(Introduced by A. V. Schally)

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It is a well-known fact that LH-releasing hormone (LH-RH) given iv induces a quick release of LH from the pituitary gland in rats and other species (1, 2). Neonatally androgenized rats are known to be sterile and bear polycystic ovaries (3). In a previous report from our laboratory it was shown that female rats androgenized neonatally with testosterone propionate responded with a higher increase of serum LH after the injection of LH-RH than normal diestrous and male rats (4). Estrogens were demonstrated to augment the pituitary response to LH-RH in terms of LH release in rats (5, 6). Since in neonatally androgenized female rats the polyfollicular ovaries might secrete estrogen continuously, these steroids could be the reason for the increased sensitivity to LH-RH in such rats. The aims of the present experiment were a) to investigate if the administration of estradiol could further increase the release of LH after the injection of LH-RH in neonatally androgenized female rats, and b) to investigate if actinomycin D was able to alter the response to LH-RH and to modify and effect of estradiol on such a response in neonatally androgenized female rats.

Materials and methods. Female rats of the Wistar strain were used. On the second day of life they were injected sc with testosterone propionate (1 mg/rat). After weaning the rats were maintained on a diet consisting of Purina laboratory chow, fresh vegetables and water *ad lib.*, with an illumination schedule of 14 hr of light and 10 hr of darkness. When the rats were 6 months old (mean weight, 200 g) they were divided into four groups of 14 rats each, according to the following treatment schedule: a) corn oil, b) estradiol dissolved in corn oil (50 μg/rat), c) estradiol (50 μg/rat) plus actinomycin D (Lyovac Cosmegen, Merck, Sharp & Dohme) dissolved in distilled water and given sc at a dose of 80 μg/100 g body wt, (the injections of estradiol and actinomycin D were given almost simultaneously at separate sites) and d) actinomycin D at the same dosage as in group c. Seventeen to eighteen hours later the rats from each group were divided into two subgroups, each one being injected iv with acidified saline or synthetic LH-RH at a dose of 100 ng/rat under light ether anesthesia. Twenty minutes later the rats were killed by decapitation, blood being collected from the trunk. Sera were separated and kept frozen until assayed. Serum LH was investigated in each serum sample by means of the double-antibody radioimmunoassay described by Niswender *et al.* (7). ^{125}I was used for labeling purifed ovine LH, an anti-ovine LH serum was used as the first antibody and NIAMDD-Rat LH-RP1 served as the standard preparation. The significance of the differences among groups was tested by means of factorial analysis and Duncan's new multiple range test (8).

Results. The pretreatment with estradiol, actinomycin D or both did not significantly modify the resting levels of serum LH, as compared with control rats pretreated with oil and injected with saline (Fig. 1). In all of the four groups LH-RH induced a highly significant increase in serum LH levels ($P < 0.01$). The magnitudes of such increases were, however, not uniform, and varied according to the different pretreatment groups. Estradiol significantly augmented LH release after the injection of LH-RH, as compared with the respective control

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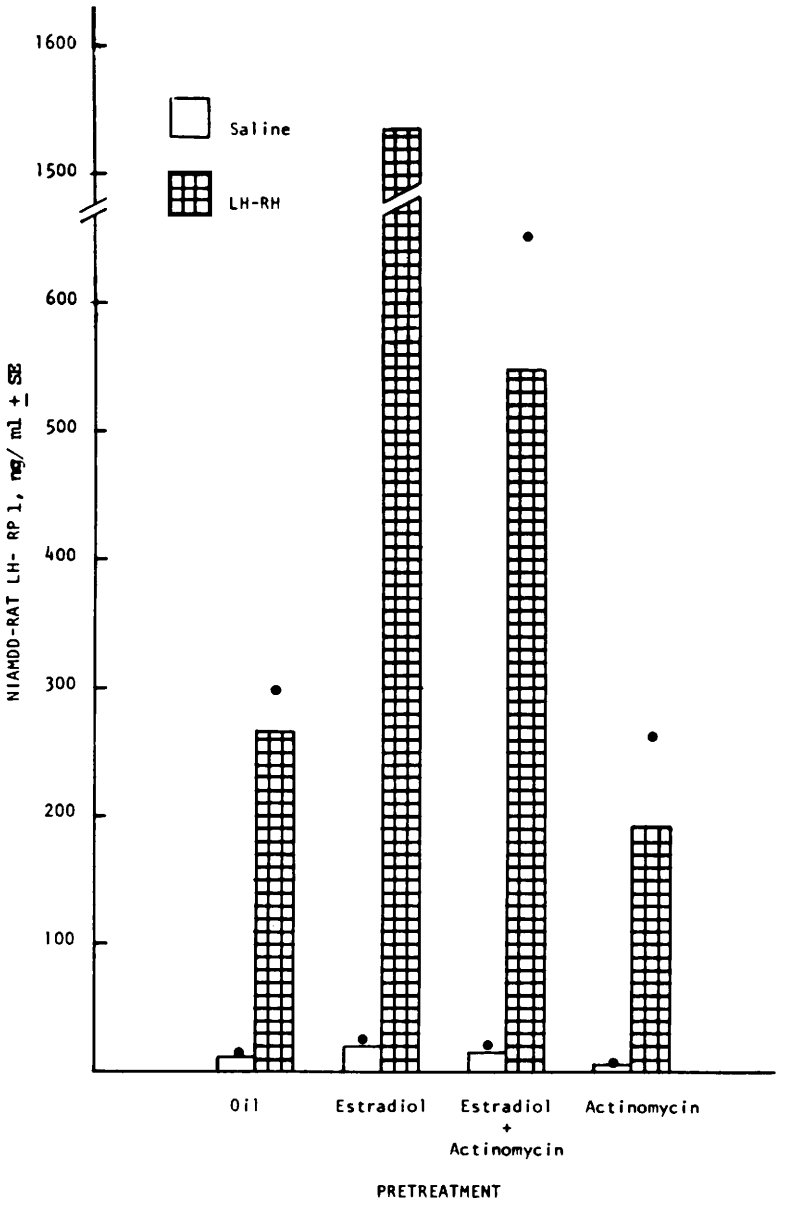

FIG. 1. Effect of the pretreatment with estradiol, actinomycin D, or both on the pituitary response to LH-RH in neonatally androgenized female rats.

group pretreated with corn oil and also injected with LH-RH ($P < 0.01$) and with similar LH-RH injected groups pretreated with actinomycin D or actinomycin D plus estradiol ($P < 0.01$). Actinomycin D given alone did not significantly modify the response to LH-RH as compared with the response to LH-RH in control rats pretreated with corn oil. In rats pretreated with actinomycin D plus estradiol the pituitary response to LH-RH, in terms of LH release, was not significantly higher than in corn oil-pretreated rats and just borderline below significance ($P = 0.05$) as compared with the response to LH-RH in rats pretreated with actinomycin D alone.

Discussion. We have previously reported that neonatally androgenized female rats

have a greater increase of serum LH after receiving LH-RH than normal diestrous and male rats (4). In spite of such higher response to LH-RH in these rats, the present experiment shows that the exogenous administration of estradiol can further increase the pituitary response to LH-RH, as was previously seen in diestrous and castrated rats (5, 9).

Actinomycin D blocks RNA synthesis (10). In the present experiment, actinomycin D did not significantly modify basal serum LH levels and the release of LH after injecting LH-RH. These two findings are in good agreement with previous data reported by other authors (11, 12). On the other hand, from the data obtained in the present investigation it is evident that actinomycin D significantly inhibited the augmenting effect of estradiol on the pituitary response to LH-RH. This fact might suggest, although it does not prove, that, directly or indirectly, protein synthesis is necessary for estradiol to exert its augmenting effect on the pituitary response to LH-RH. Previous reports had shown that actinomycin D was able to block the release of LH induced by injecting estradiol into ovariectomized rats (11) or by injecting progesterone into rats pretreated with estradiol benzoate or testosterone propionate (13). It also blocked the inhibitory effect of a combined injection of estrogen and progesterone on serum LH levels (12).

Since the effect of estradiol on the pituitary response to LH-RH was proven to be biphasic (14), with an early inhibition of such response, followed by a later augmentation of the same response to LH-RH, the present investigation shows a blocking effect of actinomycin D on the augmenting effect of estradiol. It remains to be demonstrated if such a blocking effect of actinomycin D could be exerted also on the earlier, inhibitory effect of estradiol on the pituitary response to LH-RH.

Summary. The effect of estradiol, actinomycin D, or both, on pituitary response to LH-RH was studied in 6-month-old female rats which had been injected with testosterone propionate on the 2nd day of life. Estradiol significantly augmented the pituitary response to LH-RH. Actinomycin D did not significantly modify basal serum LH levels and pituitary response to LH-RH but did significantly inhibit the augmenting effect of estradiol on such a response.

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