Effect of actinomycin D on the inhibition of the pituitary response to LH-RH by oestradiol in rats

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Recent reports have demonstrated that oestradiol modifies the pituitary response to LH-RH (Debeljuk, Arimura & Schally, 1972; Debeljuk, Vilchez Martínez, Arimura & Schally, 1974). This effect of oestradiol is biphasic, being inhibitory between 2 and 6 hr after its administration, but stimulatory after 9 hr (Vilchez Martínez, Arimura, Debeljuk & Schally, 1974). Similar results in different experimental conditions have been reported by Libertun, Orias & McCann (1974) and Van Dieten, Stejsiger, Dullaart & Van Rees (1974). We have recently shown that actinomycin D was able to block the augmenting effect of oestradiol on the pituitary response to LH-RH in neonatally androgenized rats (Debeljuk, Rettori, Rozados & Villegas Vélez, 1974). In the present report, the effects of actinomycin D were similarly investigated, but in normal dioestrous rats.

Materials and methods

Female rats of the Wistar strain (mean weight: 180 g) were used. They were maintained on a diet of laboratory chow and vegetables, and with free access to water. The light schedule was 14 hr light/24 hr. The stage of the oestrous cycle was checked every day by observation of the vaginal cytology for at least 3 cycles. Only rats with regular 4-day cycles were selected. Rats on the first day of dioestrus were separated into 4 groups of 12 animals each, and given one of the following treatments: Group 1, corn oil; Group 2, oestradiol benzoate, dissolved in corn oil, and injected s.c. at a dose of 20 µg/rat; Group 3, oestradiol benzoate (20 µg/rat) plus actinomycin D (Lyovac Cosmegen: Merck, Sharp & Dohme) dissolved in distilled water and injected s.c. at a dose of 80 µg/100 g body wt (these 2 injections were given simultaneously at separate sites); Group 4, actinomycin D, given at the same dose as in Group 3. Three hours after treatment, all the rats were lightly anaesthetized with ether and 6 animals in each group were injected i.v. with 0.2 ml acidified saline and the other 6 were injected i.v. with LH-RH, dissolved in 0.2 ml acidified saline, at a dose of 400 ng/rat. This dose of LH-RH was selected to repeat as exactly as possible the conditions of the experiment reported by Vilchez Martínez *et al.* (1974). Twenty minutes after the injection of saline or LH-RH the rats were killed by decapitation. Blood was collected from the trunk and serum was separated by centrifugation and kept frozen until assayed. Levels of LH were measured by the double-antibody method described by Niswender, Midgley, Monroe & Reichert (1968). An ovine LH preparation was used for labelling with ¹²⁵I; the first antibody was an anti-ovine LH serum, and NIAMDD-Rat-LH-RP1 was used as the standard preparation. The significance of the differences among groups was checked by means of Student's *t* test and Duncan's new multiple range test (Steel & Torrie, 1960).

Results and discussion

The administration of actinomycin D alone did not significantly modify serum LH levels in saline-injected rats as compared with the control rats pretreated with oil (Table 1). Oestradiol given alone or with actinomycin D, significantly depressed serum LH levels in the saline-injected rats, but LH-RH treatment caused significant increases of serum LH in all four groups. The lowest serum LH increase after LH-RH was observed in the rats pretreated with oestradiol.

Table 1. The inhibitory effect of oestradiol benzoate on the pituitary response to 400 ng LH-RH and its modification by actinomycin D in dioestrous rats (6/group) as shown by serum LH levels (mean $\pm$ S.E.M.)

Pretreatment	Saline-treated rats	LH-RH treated rats
Oil	11.00 $\pm$ 1.67†	567.5 $\pm$ 53.26†
Oestradiol benzoate (20 μ g)	2.33 $\pm$ 0.26*†	11.7 $\pm$ 2.21*†
Oestradiol benzoate (20 μ g) + actinomycin D (80 μ g/100 g body wt)	2.83 $\pm$ 0.29*†	265.0 $\pm$ 57.93*†
Actinomycin D (80 μ g/100 g body wt)	13.80 $\pm$ 1.26†	600.0 $\pm$ 53.07†

* Significantly different from values for oil-injected animals, $P < 0.01$.

† Significantly different from values for saline-injected animals, $P < 0.01$.

From these results it is evident that the administration of oestradiol depressed basal serum LH levels as well as the pituitary response to LH-RH in terms of LH release, and show that actinomycin D is able to block the inhibitory, as well as the stimulatory, effect of oestradiol on the response to LH-RH (Debeljuk *et al.*, 1975). These results are in good agreement with the previous report by Vilchez Martínez *et al.* (1974) that oestradiol has an inhibitory effect on LH-RH response within 2–6 hr after administration. Furthermore, the present investigation demonstrates that actinomycin D is able to reverse the blocking effect of oestradiol on the response to LH-RH. The inhibition by actinomycin D of the blocking effect of oestradiol, however, was not complete, since the rats pretreated with oestradiol plus actinomycin D showed a pituitary response to LH-RH that was significantly lower than in the control and actinomycin D-pretreated rats. Oestradiol is known to promote an early increase in protein synthesis at the translational levels with secondary augmentation of RNA polymerase activity and RNA synthesis (Gorski, 1964; Gorski, Noteboom & Nicolette, 1965; Mueller, Vondehaar, Kim & Le Mahieu, 1972). Baulieu (1974) has demonstrated a blocking effect when actinomycin D is given at the same time as oestrogen, indicating localization of the oestrogen-receptor complex at the transcriptional level. It therefore seems possible that the modulating effect of oestradiol on the response to LH-RH, which is itself modified by actinomycin D, probably also indicates an action at the translational level by inhibiting the induced protein, but we are uncertain how these events affect the release of LH. They may modify the sensitivity of gonadotrophs or may act directly on the release mechanism.

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