Oestriol-Stimulated Synthesis of Ribonucleic Acid in the Uterus of the Immature Rat

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(Received 7 November 1977)

Oestradiol-17 β and oestriol both sequentially stimulate the synthesis of heterogeneous nuclear RNA and rRNA *in vivo*. However, the stimulation by oestradiol-17 β , particularly of rRNA synthesis, is very much greater than that elicited by oestriol.

Oestriol has been shown to exhibit some of the early effects of oestrogen on the biosynthesis of macromolecules in the uterus, but to be unable to elicit later effects and the overall oestrogenic effects on uterine development. Thus, during the first 3–4 h after hormone administration, oestriol is as effective as oestradiol in inducing water imbibition (Anderson *et al.*, 1972; Lan & Katzenellenbogen, 1976), incorporation of [^{14}C]glucose into lipid, protein and RNA (Anderson *et al.*, 1972), phosphorylation of 2-deoxyglucose (Lan & Katzenellenbogen, 1976) and accumulation of hormone–receptor complex in the target-cell nucleus (Anderson *et al.*, 1972). Conversely, oestriol is not retained as well as oestrogen in the nucleus and does not produce long-term oestrogenic effects such as an increase in dry weight and overall uterine growth (Anderson *et al.*, 1972).

The effects of both hormones have also been studied with respect to their differential effects on RNA polymerase activities in isolated nuclei from ovariectomized rats (Harden *et al.*, 1976; Webster & Hamilton, 1976; Weill *et al.*, 1977). Again, both oestradiol-17 β and oestriol caused a rapid increase in polymerase II activity, which reached a peak in the first hour after hormone administration, and in the activities of polymerases I and III, which maximize at 3–4 h. Unlike oestradiol, however, oestriol did not stimulate polymerase II activity to a second peak at 4 h after treatment, nor were the peaks of polymerases I and III sustained for 12 h as during oestradiol stimulation. By that time all oestriol-stimulated activities had returned to control values.

Oestrogen treatment of ovariectomized rats can be regarded as a form of secondary exposure to hormone of animals that once produced their own oestrogen. The primary stimulation of immature animals may therefore be expected to differ in its degree and duration. Thus the immature rat responding to oestradiol shows a rapid rise in the rate of RNA synthesis, which reaches a peak at 8–12 times control values 2–4 h after hormone administration and then rapidly decreases, almost to control values by 8 h (Knowler *et al.*, 1975). This response is charac-

terized by a very rapid stimulation of heterogeneous nuclear RNA synthesis, which is a prerequisite for the later stimulation of rRNA synthesis (Knowler & Smellie, 1971, 1973). It was therefore decided to investigate whether oestriol exhibited any differential effect on the synthesis of heterogeneous nuclear RNA and rRNA in immature rat uterus *in vivo*, and the experiments here described investigate this possibility.

Materials and Methods

The source of immature rats, the administration of oestradiol-17 β and radioactive precursor, and the preparation of uterine RNA and its fractionation and detection on polyacrylamide gels have been previously described (Knowler & Smellie, 1971). Oestriol was from Sigma (London) Chemical Co., London S.W.6, U.K., and was examined for purity by g.l.c. after conversion into trimethylsilyl ether derivatives (Vandenhoeval, 1967). No oestradiol-17 β or oestrone contamination was detected at sensitivities that would have detected a contamination of 0.001%. Oestriol was administered to rats in the same manner as oestradiol-17 β , i.e. 1 μg was given intraperitoneally in 0.1 ml of 0.9% NaCl containing 0.5% (v/v) ethanol (Knowler & Smellie, 1971).

Results and Discussion

Fig. 1 compares the effects of oestradiol-17 β and oestriol on the synthesis of immature-rat uterine RNA *in vivo*. After 1 h of oestradiol-17 β treatment the main response to the hormone is a stimulation of the synthesis of very large RNA species, which have been previously shown to be heterogeneous nuclear RNA (Knowler & Smellie, 1973). At this time the rate of synthesis of rRNA and pre-ribosomal RNA species is also slightly increased, though at 30 min after hormone administration only the synthesis of heterogeneous nuclear RNA is stimulated (results not shown). By 4 h after the administration of oestradiol-17 β , there is a striking stimulation of the synthesis of all RNA species, particularly rRNA. Fig. 1 also shows that

oestriol exhibits a very similar qualitative pattern of stimulation to oestradiol-17 β in that the synthesis of heterogeneous nuclear RNA is significantly stimulated 1 h after hormone treatment, followed at 4 h by an increase in the rate of rRNA synthesis. However, the degree of stimulation, particularly of rRNA synthesis, is very much less.

Oestrogens also stimulate imbibition of water into the uterus and an associated increase in the uptake of small molecules, including RNA precursors. However, the changes recorded in the synthesis of RNA are much greater than the increased uptake of precursor and cannot be attributed to this alone (Knowler *et al.*, 1975).

These results are consistent with the concept that oestriol acts as a weak oestrogen, which, although able to associate with the receptor and move into the nucleus, is defective in its nuclear retention and oestrogenic stimulation of transcription.

I am grateful to Mrs. Anne Kelly under the direction of Dr. B. Cook of the Steroid Biochemistry Laboratory of

the Royal Infirmary, Glasgow, for performing the g.l.c. analysis of oestriol.

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Fig. 1. Separation of high-molecular-weight uterine RNA on polyacrylamide gels

Rats (18–21 days old, 25–30 g) received 1 μ g of hormone, or carrier, by intraperitoneal injection at various times before death and 125 μ Ci of each of [5- 3 H]uridine and [8- 3 H]guanosine by intravenous injection 1 h before death. Purified uterine RNA was separated by electrophoresis for 5 h on 2.7% polyacrylamide gels. After electrophoresis at 5 mA/gel the gels were scanned at 260 nm with a gel-scanning attachment for the Gilford 240 spectrophotometer. They were then sliced into 1 mm sections, which were digested with 2 M-NH $_3$ and counted for radioactivity in toluene/2-methoxyethanol-based scintillator with efficiencies of 16–18%. (a) Control; (b) 1 h after oestradiol-17 β administration; (c) 4 h after oestradiol-17 β administration; (d) 1 h after oestriol administration; (e) 4 h after oestriol administration; —, A_{260} ; ----, radioactivity (d.p.m.)/slice. For reasons of clarity the A_{260} trace is omitted for the first few slices of the gels. The position and identity of 45S and 32S RNA have been determined by methylation and co-fractionation with 14 C-labelled HeLa-cell nucleolar RNA as previously described (Knowler & Smellie, 1971).