ACUTE EFFECTS OF ACTINOMYCIN D ON THE BINDING OF TRITIATED OESTRADIOL BY THE MOUSE VAGINA*

By G. M. STONE†

Abstract

Mice received an intravaginal injection of oestradiol-17β (E_2) followed at varying times by an intravaginal injection of actinomycin D. The binding of [^{3}H]oestradiol-17β ([^{3}H]E_2) by whole vaginal tissue *in vivo* and by subcellular fractions of vaginal homogenates *in vivo* and *in vitro* was measured in animals which were killed 12 hr after the E_2 injection or which received intravaginal [^{3}H]E_2 at this time and were killed 1 hr later. Oestradiol pretreatment decreased the total tissue retention of [^{3}H]E_2 and the binding in the soluble fraction but did not change the subcellular distribution of radioactivity. With increasing time after actinomycin there was a significant linear decrease in the above parameters. The results suggest that in the mouse vagina, as opposed to the rat uterus, the process by which the level of soluble oestrogen receptor returns to that observed prior to oestradiol administration is dependent on RNA and protein synthesis.

Introduction

In the ovariectomized mouse the administration of actinomycin D 25–27 hr prior to an intravaginal injection of [^{3}H]oestradiol-17β decreased to approximately 50% the binding of the oestradiol by the vagina (Stone 1971; Stone and Pollard 1973). The net half-life of the vaginal oestrogen receptor was estimated at $24 \cdot 2 \pm 3 \cdot 2$ (S.D.) hr, a value similar to that reported by Gorski and Notides (1969) for total soluble proteins of the rat uterus but differing from the estimate, by Sarff and Gorski (1971), of 5–6 days for the half-life of the soluble oestrogen receptor from that organ. Sarff and Gorski (1971) also showed that, following oestrogen administration, the oestradiol binding ability of the soluble fraction of the rat uterus was decreased. A return to pre-injection levels was prevented by inhibitors of protein or RNA synthesis only when these antagonists were administered at about the same time as the oestrogen, suggesting that replacement of the soluble receptor under these conditions does not necessarily require new protein synthesis.

The apparent differences in the regulation of oestrogen receptor levels in the mouse vagina and rat uterus have been examined further in the experiments of this paper. The acute effects of actinomycin D on the retention of oestradiol by the mouse vagina and on the replenishment of the soluble receptor following an injection of oestradiol were studied. The dose and route of administration of actinomycin D resulted in no obvious harmful systemic effects and have previously been shown apparently not to alter the morphology of the epithelial cells (Pollard 1970).

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† Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W. 2006.

Materials and Methods

(i) *Solutions.*—[2,4,6,7-^{3}H]oestradiol-17β (specific activity 370 μCi/μg) was obtained from the Radiochemical Centre, Amersham, England. The purification before use and preparation for intravaginal injection and *in vitro* incubation have been previously described (Stone and Baggett 1965; Stone and Pollard 1973). Actinomycin D (Calbiochem) was dissolved in distilled water to give the required level for injection, 1·2 μg, in 10 μl.

(ii) *Animals and Experimental Treatments.*—Randomly bred mice of the QS strain were ovariectomized, primed, and randomized to experimental groups as previously described (Stone 1971). Two experiments were done, each with basically the same treatments. Mice received 25 pg of oestradiol-17β (E_2) intravaginally 12 hr prior to killing or prior to an intravaginal injection of 25 pg of [^{3}H]oestradiol-17β ([^{3}H]E_2). These latter animals were killed 1 hr after the injection of the [^{3}H]E_2. Actinomycin D was administered intravaginally 10, 8, 6, or 4 hr before killing or before the [^{3}H]E_2 injection (treatments B, C, D, and E respectively). In one control treatment (A) no actinomycin D was administered and in another (G) neither actinomycin D nor the initial oestradiol were administered. In experiment 1 each treatment comprised a group of five mice which received [^{3}H]E_2 and were killed 1 hr later. The radioactivity in the individual vaginae was measured (Stone 1971). In addition each treatment comprised four groups each of which contained pooled tissue from five mice which had received no [^{3}H]E_2. In these latter the oestrogen-binding ability of the 105,000 *g* supernatant fractions, prepared from vaginal homogenates, was studied *in vitro* by charcoal absorption (Stone and Pollard 1973). Replicate supernatant aliquots, representing tissue from two mice, were incubated at 4°C with 25 pg of [^{3}H]E_2 for 3 hr prior to the addition of the charcoal–dextran. In experiment 2 each treatment comprised four groups each of five mice which were killed 1 hr after the [^{3}H]E_2. Radioactivity in pooled 105,000 *g* supernatant and pellet fractions and the oestrogen binding in the supernatant were measured (Stone 1971; Stone and Pollard 1973).

Results

Table 1 shows the results of both experiments. Oestradiol pretreatment did not affect the subcellular distribution of radioactivity as studied but did decrease the total tissue retention and thus the proportion of the injected radioactivity associated with each tissue fraction. The oestradiol binding in the supernatant following *in vivo* injection or *in vitro* incubation was also decreased. With increasing time of exposure to actinomycin D there was a significant linear decrease in the above parameters and these, at no time, significantly exceeded the values attained in animals which had received oestradiol but not actinomycin D.

Discussion

These experiments indicate that following the administration of oestradiol the return of the oestrogen-binding ability of the soluble fraction of mouse vaginal homogenates to pre-injection levels is dependant on RNA and subsequent protein synthesis. This conclusion appears to differ from and be rather more simplistic than that of Sarff and Gorski (1971) who carried out similar studies with the rat uterus. The design of the present experiments does not allow a differentiation between an effect of actinomycin D on new receptor synthesis or an effect on any mechanism involved in the recycling of the receptor between the nucleus and the soluble fraction. Previous studies (Stone 1971; Stone and Pollard 1973) have showed that, at later times, actinomycin D decreased the total level of receptor in the tissue and the transfer of oestradiol from the soluble fraction to the nucleus, suggesting that both actions might be involved in the mouse vagina.

TABLE 1

EFFECT OF ACTINOMYCIN D ON THE BINDING OF [^{3}H]OESTRADIOL-17β ([^{3}H]E_2) BY THE MOUSE VAGINA FOLLOWING THE *IN VIVO* ADMINISTRATION OF UNLABELLED OESTRADIOL-17β (E_2)

Mice received an intravaginal injection of E_2 (25 pg) followed at the times indicated by an intravaginal injection of actinomycin D (1·2 μg). The binding of [^{3}H]E_2 by whole vaginal tissue *in vivo* and by subcellular fractions of vaginal homogenates *in vivo* or *in vitro* was measured in animals which were killed 12 hr after the E_2 injection or which received intravaginal [^{3}H]E_2 (25 pg) at this time and were killed 1 hr later

Treatment code	E_2 12 hr before	Actinomycin D pretreatment time	Experiment 1		Experiment 2			
			Total in fractions (% of injected)	Cytosol fractions binding (% of incubated)	Total in fractions (% of injected)	Nuclear fraction (% of injected)	Nuclear fraction (% of recovered)	Cytosol fraction binding (% of incubated)
A	+	—	51·9	36·1	57·8	32·7	56·3	58·9
B	+	10	41·7	21·1	43·3	22·4	51·6	42·4
C	+	8	43·0	29·6	45·2	24·8	54·7	45·2
D	+	6	44·8	31·4	48·8	29·9	61·2	48·8
E	+	4	47·3	37·1	51·8	30·4	58·7	59·9
G	−	—	73·4	52·4	78·5	45·2	57·3	70·4

Summary of analyses of variance

Source of variation	Expt. 1: Variance ratios†				Expt. 2: Variance ratios			
A *v.* B+C+D+E	17·31***	(1)	6·05*	(1)	22·8***	7·46*	0·01	22·47***
Time of actinomycin D								
Linear	6·31*	(1)	23·97***	(1)	10·99**	11·81**	4·61*	45·64**
Quadratic	0·12	(1)	0·31	(1)	0·07	0·23	1·69	4·96*
Cubic	0·00	(1)	1·06	(1)	0·08	0·95	1·66	0·63
Within group	*13·70*	(20)	*20·8*	(15)	*15·62*	*14·39*	*17·72*	*13·89*

* $0·05 > P > 0·01$. ** $0·01 > P > 0·001$. *** $P < 0·001$. † Number of degrees of freedom given in parenthesis.

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