Biological Actions of Estradiol-17β After Prolonged Ovarian Hormonal Deficits

The literature is replete with short, critical experiments involving the biological actions and inter-actions of a) the estrogens, singly and in combination, b) the progestational hormones, likewise singly and in combination, and c) the estrogens and progestins in a number of different combinations or physiological ratios on the reproductive tracts of a number of different species of laboratory mammals[1-7]. The uterine growth promoting effects of a large number of estrogens and progestins thus appeared to be well-known, including the modulating[2], depressing[3], inhibiting[4], restricting[5-7] and suppressing[8,9] characteristics of these C-18 and C-21 ovarian sex steroid hormones. The point naturally emerged to ascertain the status of the uterine growth response in adult, albino rats which were ovariectomized for different periods of time, and which were injected with sex steroid hormones for extended periods of time. In an effort to obtain some additional worthwhile base line data, it appeared of interest to first observe the effects of an estrogen in rats which had been bilaterally ovariectomized for periods of time in excess of 1 year. In this way, one could obtain quantifiable data on the effects of estrogen in rats which manifested cellular and tissue effects resulting from prolonged hormonal deficits.

Materials and methods. Descendants of the Sprague-Dawley strain of rats were utilized in these experiments. They were fed Purina Lab Chow and water ad libitum. All rats were 9-month-old adult females, at the time of surgery. 44 animals were bilaterally ovariectomized, and an additional 11 were sham-operated. $15^1/_2$ months post-ovariectomy, the animals were placed in 5 different groups, and were subcutaneously injected once daily for 3 days. The 5 groups were so arranged: Group I, sham-operated; Group II, ovariectomized and given the vehicle, 0.1 cm^3 sesame oil once daily for 3 days; Groups III, IV and V were also ovariectomized and were given 0.1 µg, 1.0 µg, and 5.0 µg estradiol-17β daily for 3 days, respectively. Necropsies were performed 24 h after the last administered injection. The uteri and endocrine organs were quickly removed, cleanly dissected and carefully trimmed free of adhering tissues, weighed to the nearest 0.1 mg on a Roller-Smith torsion balance, and fixed for subsequent cytochemical, histochemical and histologic analyses.

Results and discussion. First, and of paramount significance it should be emphasized that histological observations of the uteri did not reveal any abnormal or pathological change. The uteri, especially the glandular and luminal epithelia revealed the usual stimulatory patterns of response to estradiol-17β.

Secondly, the gravimetric analyses, cf. tabular data, revealed quite conclusively that a) ovariectomy for prolonged periods of time, i.e. $15^1/_2$ months, elicited marked ovariectomy-induced changes: whereas the uteri of the intact control rats averaged 630.1 $\pm$ 84.6 mg, those of the sesame oil, hormonal vehicle-treated ovariectomized rats weighed 74.2 $\pm$ 3.6 mg; b) sesame oil does not induce uterine weight increase, nor did it show any mitogenic activity when studied histomorphologically; c) 0.1 µg estradiol-17β daily for 3 days, given to rats previously ovariectomized for $15^1/_2$ months, proved remarkably potent in uterine-growth promoting activity, a 92% increase being observed; d) increasing the daily dosage of estradiol 10 times (to 1.0 µg daily for 3 days) elicited a more dramatic increase, 207% over the sesame oil treated ovariectomized controls, and e) further increasing the dosage 50 times, i.e. to 5.0 µg daily for 3 days, gave a further increase, 231% over the non-estrogenized group. Thus, these gravimetric analyses provide unequivocal data clearly indicating that the uterine growth response remains quite active following periods of prolonged ovarian hormonal deficits as have been observed in rats so treated following periods of ovariectomy for as long as $15^1/_2$ months.

Comparative gravimetric data at hand derived from similar experiments, but commencing estrogenization 1 week after bilateral ovariectomy in 50-day-old rats revealed that when animals were similarly given 0.1, 1.0 and 5.0 µg estradiol-17β daily for 3 days and necropsied 24 h after the last injection, with all protocols of a precisely duplicate nature, the uterine increases were 88, 120 and 136%, respectively, over those of the sesame oil-treated, ovariectomized controls (VELARDO[8]). This contrasts sharply with the marked, dramatic increases herein observed on the present experiments, i.e. 92, 207, and 231%, respectively, on the same dosages. Thus, these data extend an earlier report by VELARDO and KASPROW[10] indicating that it appears satisfactory to utilize animals

Influence of estradiol-17β on the uteri of adult, albino rats $15^1/_2$ months after bilateral ovariectomy

Group	Body wt. (g)	Uterine wt. (mg)
I. Non-Rx; sham-operated (intact controls)	309.4$\pm$11.5[b]	630.1$\pm$84.6
II. ♀, +0.1 cm^3 sesame oil (ovariectomized hormonal-vehicle controls)	319.3$\pm$ 3.6	74.2$\pm$3.6
III. ♀, +0.1 µg est. 17β[a] daily for 3 days	334.4$\pm$ 4.5	142.4$\pm$13.8
IV. ♀, +1.0 µg est. 17β daily for 3 days	363.8$\pm$ 7.6	227.8$\pm$17.8
V. ♀, +5.0 µg est. 17β	394.1$\pm$ 3.7	245.7$\pm$15.9

[a] Est. 17β, estradiol-17β (estra-1, 3, 5 (10)-triene-3, 17β-diol).
[b] $\pm$, standard error of the mean.

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of this age to ascertain the changes in the nature of the several cellular components that figure importantly in uterine growth[11].

Riassunto. Si dimostra che l'utero di ratta, sottoposta ad ablazione della ovaie 15 mesi e mezzo prima, risponde nettamente a tre iniezioni sottocutanee quotidiane di 17β-estradiolo: si ottennero aumenti di peso del 92, 207 e 231% al di sopra del peso degli uteri di controlli sottoposti ad ovariectomia e trattati con semplice olio di sesamo. Si conclude, percio, che l'utero di ratta esposta a deficienza ormonica ovarica di lunga durata è notevolmente sensibile a trattamento con estrogeni.

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Evidence for a Hypocalcemic Factor in the Hypothalamus

The hypocalcemic effect of the pituitary gland extracts have been shown by several workers[1-5]. So far, there is no convincing evidence that the hypothalamus is concerned in the production of hypocalcemia. The present study suggests the existence of a hypocalcemic factor in the hypothalamus.

Material and methods. 72 albino rats of Hacettepe strain, weighing 150 to 180 g and 470 guinea-pigs were used in this study. All rats were placed on a special low calcium diet for 3 days before the experiment. Fragments of hypothalamic tissue, containing mainly pituitary stalk and median eminence (SME), and cerebral cortex in an equivalant amount to SME were removed from 470 guinea-pigs immediately after decapitation, frozen on dry ice and were kept for a maximum of 24 h. The 2 pools, SME and cerebrum, were thawed and each one homogenized in 25 ml chilled 0.9% saline solution. The crude homogenates were then subjected to centrifugation at 11.000 *g* for 5 min at 4 °C and the supernatants were separated. The procedures were repeated once more with 15 ml of normal saline and total extracts were kept at −20 °C. The extracts were used within 10 days.

Hypophysectomy was performed according to the method of FALCONI et al.[6]. The experiment was started the day after hypophysectomy. Rats were anesthetized with i.p. pentobarbital, 30 mg/kg of body weight prior to the experiments, after which tracheostomy was performed. A fine polyethylene catheter was inserted into the right jugular vein for extraction of blood. Brain or SME extracts or saline was injected through the left carotid artery. 1 mg of heparin was administered i.v. to each animal to prevent clotting of the blood. 1 ml of blood was drawn at O 'base line' after 20 and 30 min and in 1 experiment at 60 min for calcium determination. Each time fluid loss was replaced by infusing normal saline. Plasma calcium was determined by the method of REHELL[7]. All samples were run in duplicate.

The following 4 different experiments were carried out in this study: Experiment I 27 rats were used in this experiment. First 1 ml of blood was obtained from each intact rat and then the animals received a single 0.5 ml SME extract/100 g body wt. by carotid artery over 2 min. 15 control rats received only normal saline 0.5 ml/100 g body wt. by carotid artery. 1 ml of blood was drawn at 0, 20 and 30 min for Ca determination.

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Plasma Ca levels before and after injection of SME extract or physiological saline

Experiment	No. of rats	Infused solutions	Plasma Ca levels mg/100 ml (mean ± SE)			
			Preinjection (min)		Postinjection (min)	
			0	20	30	60
I	27	Hypothalamic (SME) extract	10.48±0.081	8.37±0.182 P<0.001[a]	8.35±0.178 P<0.001[a]	—
	15	Physiologic saline	10.39±0.100	10.10±0.090 P>0.05	10.19±0.094 P>0.05	—
II	10	Cerebral Cortex	10.94±0.168	—	10.92±0.162 P>0.05	10.97±0.189 P>0.05
III	10	Hypothalamic (SME) extract	10.78±0.288	9.56±0.302 P<0.001[a]	9.19±0.320 P<0.001[a]	—
IV	5	Heated (SME) extact	10.80±0.423	10.88±0.312 P>0.05	10.82±0.353 P>0.05	—
	5	Digested (SME) extract	11.00±0.221	10.60±0.299 P>0.05	10.62±0.312 P>0.05	—

a — $P < 0.001$ as compared with saline infused control.