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PITUITARY UTEROTROPHIC EFFECT IN THE ESTROGEN-DEPENDENT GROWTH OF THE RAT UTERUS

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SUMMARY

Several groups of endocrine manipulated prepuberal and young adult female rats were studied with the purpose of estimating the pituitary contribution to the uterine growth after a single injection of a physiological dose (1 μg/kg of body weight) of estradiol-17β (E_2). The different groups of E_2 and vehicle-injected animals included (a) castrated and (b) hypophysectomized and castrated (hypox) animals. In addition to these groups, a third represented by (c) hypox young adult female $C_8$11RAP pituitary tumor-bearing rats were also studied to check whether this tumor behaves as the *in situ* pituitary present in castrated-only female rats. Results indicate that the pituitary of castrated rats and the rat pituitary tumor growing in the hypox rats significantly influence the increase of the wet and dry weights of the uterus following E_2 stimulation. It is proposed that there are uterotrophic factors in the pituitary and in the rat pituitary tumor tested.

INTRODUCTION

It has been suggested that estradiol-17β (E_2) effects on target cells are mediated by the interaction of this sex steroid with high affinity saturable receptors present in the cytosol of those target cells which, under well defined conditions translocate into the nucleus[1, 2]. Once in the nucleus, either the hormone, the "transformed" receptor, or the E_2–receptor complex would supposedly elicit the growth and metabolic effect from these cells that has been attributed to E_2. A number of models designed to explain this interaction at the molecular level of transcription and/or translation have been proposed[3–7]. However, results from experiments on live animals and those coming from *bona fide* estrogen target cells in short and long-term growing conditions cannot be satisfactorily integrated in a scheme that would explain all the accumulated information in these diverse systems. For example, no differential growth stimulation by E_2 was demonstrated in culture conditions of mouse vagina[8] human endometrium[9, 10], rat endometrium[11] and rat pituitary cell lines[12, 13] among others. Similar conflicting reports have been documented for the putative E_2 induction of some proteins in cultured E target cells[14, 15].

Most likely, the effect of E_2 on the uterus may be the summation of action of E_2 *per se* plus that interaction of E_2 on target organs, other than the uterus, which may be under direct and/or indirect estrogenic influence. The hypothalamo-pituitary axis is affected by changing sex steroid concentrations in the bloodstream at the same time when these same compounds (sex steroids) produce important changes in other target organs such as the uterus, vagina, mammary glands, etc.

A review of the literature on the subject of mechanism of action of estrogens reveals that most reports designed to determine the effect of E_2 on the uterus, utilize castrated animals in order to eliminate the masking effect of endogenous E_2. Several authors have briefly mentioned however, the existence of a "pituitary contribution" to the E_2 effect on the uterus[16,17].

Experiments were designed to study the direct and indirect estrogenic response at the uterine level in (a) 28 day-old castrated and/or hypophysectomized rats operated 7 days prior to being injected with E_2, (b) adult female rats subjected to the same endocrine manipulation, and (c) pituitary-tumor bearing rats operated and treated identically as those in groups (a) and (b). In this communication we are reporting the effect of E_2 on the wet and dry uterine weights of these rats. The pituitary seems to contribute significantly to the total response attributed to E_2 stimulation in castrated animals.

MATERIALS AND METHODS

Several experiments were performed with hypophysectomized and castrated animals. Hypophysectomy was performed through a ventral approach. Hypophysectomized and castrated animals will be called hypox for convenience. All sacrificed animals were checked for the presence of remnants of pituitary tissues by opening and checking the base of the skull. Whenever a suspected piece of tissue was found, a histologic survey was conducted and if pituitary tissue resulted from such case, it was dismissed from the population surveyed.

Experiment I

Hypophysectomies and/or oophorectomies were performed in 21 day-old Sprague–Dawley female rats and sent to our animal farm 4 days after the operation by Hormone Assays Laboratories, Chicago, IL. Animals were sacrificed at 28 days of age, that is, 7 days after the operation. Estradiol-17β (E_2) from Sigma Co., Saint Louis, MO was injected s.c. at a dose of 1 μg/kg of body-weight 24 h before the rats were sacrificed. As controls, vehicle-injected animals were used. Animals were sacrificed by decapitation, with a minimum of stress. E_2 was injected for this experiment and for those described below in a solution of 1% alcohol in saline. A solution of this same type without E_2 was injected into the control group of animals.

Experiment II

Hypox adult female Wistar/Furth rats (Microbiological Associates, Rockville, MD, U.S.A.) were injected separately with ovine prolactin (NIH-P-S-11) 10 mg/kg body weight; ovine Luteinizing Hormone (NIH-LH-518) 1 mg/kg body weight; ovine Follicle Stimulating Hormone (NIH-FSH-S-10) 1 mg/kg body weight; and ovine Growth Hormone (NIH-GH-S-11) 10 mg/kg body weight. All these hormone preparations were obtained through the USPHS Pituitary Hormone Program NIAMD to which we are grateful.

We have also injected each of the above mentioned ovine pituitary hormones in addition to E_2 at the same 1 μg/kg body weight doses we used for Experiment I, always 24 h before sacrificing the rats.

Experiment III

Experiments designed to test and quantify the "pituitary contribution" to the overall response of the uterus to E_2 in rats were performed. In addition to the 10–12 week old castrated and castrated-hypox Wistar/Furth rats, similar animals bearing a pituitary transplantable tumor were tested. The rationale for using this transplantable pituitary tumor-bearing group of rats was to test whether these tumors are capable of behaving as normal pituitaries under E_2 stimulation. Further, by using this group of hypox pituitary tumor-bearing animals the intermediary role played by the hypothalamus when E_2 is injected in intact animals is eliminated. The experimental design used here supposedly would have measured the direct effect of E_2 on these pituitary tumor cells. Special interest is assigned to these tumors when, as discussed below, they could be used as material from which uterotrophic factor(s) could be extracted and characterized with greater ease than from normal E_2 stimulated pituitaries. Briefly, the $C_8$11RAP tumor is one of a series of rat pituitary tumors that grow in rats when injected s.c. or i.m. in Wistar/Furth strain rats[12]. These rat pituitary tumors have a distinctive growth advantage when injected into animals where an endogenous or exogenous supply of estrogens is available. Because of this, experiments where $C_8$11RAP transplants are grown in prepuberal rats are difficult to perform and compare with adequate controls. Thus, it was decided to run this experiment in young adult female rats under comparable conditions. In these female animals, $C_8$11RAP transplanted tumors reach a 1.0 to 1.5 cm in diameter about a month after s.c. or i.m. injection of about 10^6 cells[12]. When the $C_8$11RAP tumors reached a size of 1 cm in diameter, the animals were hypophysectomized and castrated. Simultaneously, normal animals of the same age were hypophysectomized and/or castrated. One week after surgery, one half of each group received a s.c. injection of E_2 (1 μg/kg body weight). The other half received a similar volume of vehicle. All the animals were sacrificed by decapitation 24 h after the injection of E_2 or the vehicle.

Uteri from animals used in all these experiments were carefully dissected to eliminate surrounding fat and extraneous material. Fluid present in the uterine lumen was eliminated by cutting the body of the uterine horns and blotting under slight, but firm pressure. Uteri were placed on tared plastic trays and weighed on a Semimicro Mettler H 34 balance. This first measurement constituted the wet weight. These uteri in their respective trays were then placed in an incubator at 37°C and weighed at 24 h intervals until the uteri reached a constant weight. These values constitute the "dry weights".

Statistical significance was determined by Student's t-test and by analysis of variance[18].

RESULTS AND DISCUSSION

Experiments with 28 day-old and young adult female rats

Table 1 shows the average uterine wet and dry weights ($\pm$ one standard deviation) obtained 24, 48 and 72 h after E_2 injection and in controls. The wet and dry uterine weights of the castrated controls were higher than those observed in the control hypox animals ($P < 0.02$). The E_2 treatment increased the wet and dry uterine weights in both experimental groups; however, the wet and dry uterine weights of E_2-injected castrates were significantly higher than those of the E_2-injected hypox animals. The increase in uterine wet and dry weights, expressed by the ratio $\Delta W/W_0$ (E_2 treated minus control uterine weight/control uterine weight) was significantly higher in castrated than in hypox animals when determined at 24 and at 48 h after E_2-injection (Table 2), that is, when the uteri in both groups were at their maximal wet weights (Table 1).

In the experiments done with young adult females, on the other hand, the difference in $\Delta W/W_0$ between the hypox and the castrated rats is less evident (Table 2 and 4). The difference in the magnitude of the contribution by the pituitary of prepuberal and adult animals subjected otherwise to the same endocrine manipulation is not readily explained. Evidence indicating that no significant difference in estrogen receptor con-

Table 1. Wet and dry uterine weights after E_2 injection in hypophysectomized-castrated and castrated 28 day-old rats (mean ± standard deviation: Uterine weight is expressed in mg)

	Wet wt of uteri			Dry wt of uteri		
	24 h	48 h	72 h	24 h	48 h	72 h
Hypox C	14.8 ± 2.5	13.6 ± 0.96	12.4 ± 1.06	2.8 ± 0.55	2.95 ± 0.46	2.7 ± 0.22
Hypox E_2	21.73 ± 3.6	23.7 ± 2.98	17.1 ± 2.94	3.8 ± 0.60	4.50 ± 0.52	3.5 ± 0.89
	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.02$	$P < 0.005$	$P < 0.005$
Castr. C	18.2 ± 2.68	19.0 ± 0.84	19.9 ± 1.74	3.9 ± 0.55	4.0 ± 1.05	4.0 ± 0.49
Castr. E_2	37.6 ± 7.2	40.7 ± 7.48	31.9 ± 7.0	6.8 ± 0.82	7.2 ± 0.80	5.9 ± 1.17
	$P < 0.001$	$P < 0.005$	$P < 0.005$	$P < 0.001$	$P < 0.002$	$P < 0.01$

Table 2. Growth ratio ($\Delta W/W_0$) from data in wet and dry uterine weight after E_2 treatment on hypophysectomized-castrated and castrated 28 day-old rats

	Wet wt		Dry wt	
	24 h $\bar{x}$ ± S.D.	48 h $\bar{x}$ ± S.D.	24 h $\bar{x}$ ± S.D.	48 h $\bar{x}$ ± S.D.
Hypox	0.440 ± 0.1	0.732 ± 0.28	0.376 ± 0.30	0.551 ± 0.29
Castrated	0.905 ± 0.2	1.105 ± 0.30	0.950 ± 0.25	0.822 ± 0.26
P value	<0.001	<0.01	<0.005	<0.02
Ratio C/H	2.05	1.51	2.53	1.50

tents between uteri of adult castrated and prepuberal animals has been reported[19, 20]. The presence of higher values of α-fetoprotein in extracellular components of these two different groups of rats may play a role in the growth promoting effect directly or indirectly elicited by E_2[21].

To test whether the known pituitary hormones may account for the difference in weight of the uterus of castrated versus hypox females, ovine prolactin, LH, FSH and GH were injected separately into hypox animals (Experiment II). In this experiment and when E_2 was injected simultaneously with a pituitary hormone, no significant increase in uterine wet weight was observed when compared with E_2 alone. Lack of a complementary effect on uterine wet weight by pituitary hormones injected i.v. into hypox rats may reflect the short biological half-life of such hormones[22]. Alternatively, as the results of these treatments were measured 24 h after the administration of the hormones tested, the possibility that their effect may have been maximal at another time cannot be ruled out. A third explanation may be that uterus is not a "target" for these hormones. A combination of these possibilities may in fact be responsible for the results obtained.

Experiments in E_2-stimulated pituitary tumor-bearing rats

Tables 3, 4, and 5 describe and analyze the data of Experiment III. Estradiol treatment increased both wet and dry uterine weight in castrated, hypox and $C_8$11RAP tumor-bearing hypox rats compared to

Table 3. Wet and dry uterine weights and growth ratios after E_2 treatment of castrated, hypox and $C_8$11RAP tumor-bearing young adult rats

	Groups of rats	Wet wt $\bar{x}$ ± S.D.(*)	*t*	*P*	$\Delta W/W_0$	Dry wt $\bar{x}$ ± S.D.	*t*	*P*	$\Delta W/W_0$
I	Castrated + saline	138.4 ± 8.7 (10)				30.62 ± 2.02 (7)			
			9.50	<0.0005	0.38		4.77	<0.0005	0.225
II	Castrated + E_2	190.4 ± 15.1 (10)				37.50 ± 2.90 (7)			
III	Hypox + saline + $C_8$11RAP tumor	125.1 ± 15.1 (10)				26.70 ± 3.29 (7)			
			8.90	<0.0005	0.50		5.65	<0.0005	0.349
IV	Hypox + E_2 + $C_8$11RAP tumor	187.8 ± 15.6 (13)				35.95 ± 3.02 (10)			
V	Hypox + saline	111.8 ± 13.5 (10)				25.41 ± 1.9 (7)			
			3.30	<0.005	0.32		3.88	<0.0025	0.216
VI	Hypox + E_2	148.04 ± 21.7 (14)				30.92 ± 3.14 (11)			

* Indicates the number of animals tested in each group.

Table 4. Analysis of variance of the data presented in Table 3

	Analysis of variance			
	Wet weight		Dry weight	
	F ratio	Theoretical F ratio $P = 0.05$	F ratio	Theoretical F ratio $P = 0.05$
Among saline treated groups	$F_{2,27} = 24.9$	3.35	$F_{2,18} = 8.27$	3.55
Among E_2 treated groups	$F_{2,34} = 25.4$	3.28	$F_{2,25} = 4.13$	3.39
Among the 6 groups	$F_{5,61} = 97.6$	2.37	$F_{5,43} = 9.50$	2.44

that obtained in the control groups injected with vehicle alone (Table 3). The analysis of variance of the data obtained for both wet and dry weights showed that the three control groups as well as the E_2-treated groups belong to different populations (Table 4).

The comparison between pairs of control groups using the Student's t-test showed that under these conditions the uteri from the hypox rats and those from the tumor-bearing hypox rats are not significantly different, while the uteri from the castrated animals were heavier than the uteri from hypox animals. When the E_2-treated groups were compared, the uterine weight in castrated rats was significantly higher than the value obtained from hypox animals, and not significantly different from those found in E_2-treated $C_8$11RAP tumor-bearing rats (Table 5).

Table 5. Comparison through the t-test of the uterine wet and dry weights among the different vehicle-treated groups and among the E_2-treated groups

	Wet weight		Dry weight	
Groups of rats	t	P	t	P
Castrated + saline vs Hypox + saline	5.01	<0.0005	4.58	<0.0005
Castrated + saline vs Hypox + saline + $C_8$11RAP tumor	2.51	<0.0125	2.47	<0.025
Hypox + saline vs Hypox + saline + $C_8$11RAP tumor	1.97	NS	0.83	NS
Castrated + E_2 vs Hypox + E_2	4.84	<0.0005	4.21	<0.0005
Castrated + E_2 vs Hypox + E_2 + $C_8$11RAP tumor	0.36	NS	0.99	NS
Hypox + E_2 vs Hypox + E_2 + $C_8$11RAP tumor	5.20	<0.0005	3.05	<0.005

In Table 3, a sizeable difference in growth rate can be seen when the hypox groups of animals (V and VI) are compared with the hypox tumor-bearing animals (III and IV). While the $\Delta W/W_0$ for Groups V and VI is 0.32 and 0.216 for wet and dry weights, respectively, it is 0.50 and 0.34 for wet and dry weights, respectively, for groups III and IV. These results lead us to believe that the $C_8$11RAP tumor secrete uterotrophic factor(s) responsible for such differences in the growth capacity of the uteri seen in the castrated groups of animals. Further, the results of Experiment III suggest that the E_2-mediated response described may not require the participation of the hypothalamus to be elicited at the pituitary level.

Based on the evidence that the known pituitary hormones injected separately or in association with E_2 into hypox animals do not show an increase in uterine wet weight similar to that seen in castrated animals stimulated with E_2 or of the E_2-stimulated $C_8$11RAP tumor-bearing hypox animals, we postulate that (an) "uterotrophic factor"(s) is(are) likely to be responsible for the difference between the wet and dry weights of uteri of Groups I, II, III and IV of Table 3.

In sum, the results of the experiments described above again point out to the existence of a paradox when the E stimulation of whole animals and of *bona fide* E target cells in culture conditions are compared. In order to reconcile these two accepted facts it has to be postulated that (1) E_2 *per se* is not able to produce the full response measured at the uterine level in castrated rats, (2) that mechanisms triggered directly or indirectly by E_2 all along the hypothalamo-pituitary-thyroid-adrenal axis play an important role to accomplish the full response seen in castrated rats. Our results indicate that the pituitary contributes to the wet and dry weight increases measured 24 and 48 hours after E_2 stimulation of castrated rats. Further studies are in progress to more precisely define the postulated E_2-dependent factors. We believe that the above mentioned paradox will be finally reconciled.

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