Effect of Steroid Hormones, Ovariectomy, Estrogen Pretreatment, Sex and Immaturity on the Distribution of ^{3}H-Estradiol

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ABSTRACT. Binding sites of limited capacity for ^{3}H-estradiol have been demonstrated in the anterior pituitary, uterus, vagina and hypothalamus. Administration of estrone or estriol decreased the accumulation of ^{3}H-estradiol in the anterior pituitary, uterus, vagina and hypothalamus, whereas the concentration of ^{3}H-estradiol in the heart, cerebrum and plasma was not reduced. High doses of progesterone, testosterone, or hydrocortisone did not reduce the concentration of ^{3}H-estradiol in tissues. These observations indicate a high specificity for attachment to the estradiol binding sites. The distribution of ^{3}H-estradiol was similar in intact and ovariectomized rats except for a lower concentration but higher content in the uterus of the animals with ovaries. Treatment of ovariectomized rats with estrogen increased the capacity of the uterus to bind estradiol. The accumulation of ^{3}H-estradiol in peripheral organs and, probably, in the central nervous system was similar in mature and immature female rats. The central nervous system of male and female rats accumulated ^{3}H-estradiol to the same extent. In contrast to the female sexual organs, the testis, epididymis and prostate did not concentrate ^{3}H-estradiol. (*Endocrinology* **79:** 38, 1966)

THE PRESENCE of specific binding sites for estradiol in certain organs has been indicated by studies with radioactive estrogen. ^{3}H-estradiol selectively accumulated in the uterus, vagina and anterior pituitary (1–3). In the central nervous system, higher retention occurred in the hypothalamus, preoptic region and septum (1, 4), and, furthermore, specific neurons in these regions highly concentrated an estrogenic compound (4). The binding capacity for estradiol of the anterior pituitary, uterus, vagina and hypothalamus was found to be limited, as evidenced by saturation with low doses of estradiol (1). Furthermore, certain drugs, such as norethynodrel, competed with estradiol for binding in these organs (1).

The present report describes ^{3}H-estradiol binding in a variety of tissues as affected by estrone, estriol, progesterone, testosterone and hydrocortisone. In addition, the distribution of ^{3}H-estradiol has been compared in female rats with and without ovaries, with estrogen pretreatment, and in male and immature female rats.

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Materials and Methods

Mature Sprague-Dawley rats, ovariectomized at least 2 weeks previously, 200 g male and 100 g immature female rats were used in the experiments. Six animals were used in each group.

^{3}H-estradiol (38 c/mmole, New England Nuclear Corporation), 0.1 μg/100 g of body wt, was injected in the tail vein and the rats were killed 1 hr later. The other steroids (Calbiochem, grade A) were injected intravenously in 0.2 ml ethanol 30 sec before the ^{3}H-estradiol; the control group received the diluent alone. ^{3}H-estradiol was measured by extraction from an aqueous suspension into an organic phase of low dielectric constant, as has previously been described (1). The organs were homogenized in 2 ml ice-cold water and the aqueous suspension was shaken with 10 ml of toluene:isoamyl alcohol (19:1) for 1 hr. An aliquot of the organic phase was then added to phosphor and counted. Over 90% of the radioactivity in the organic phase from the anterior pituitary, hypothalamus, uterus and vagina, 80% from the cerebrum and heart and 50% from the plasma are chromatographically identical with authentic estradiol.

Results

Estrone, estriol, progesterone, testosterone or hydrocortisone was injected intravenously, followed 30 seconds later by ^{3}H-estradiol. The animals were killed one hour later and the tissues were assayed for ^{3}H-estradiol (Fig. 1). Estrone and estriol markedly reduced the accumulation of ^{3}H-estradiol in the anterior pituitary, uterus, vagina and hypothalamus but had little or no effect on the concentrations in the cerebrum or heart. There was a slight increase (significant with estriol) in the plasma concentration. If it is assumed that the cerebral concentration is due to nonspecific absorption and that nonspecific absorption in the hypothalamus occurs to the same extent, accumulation in specific neurons may be better approximated by subtracting the cerebral concentration from the hypothalamic concentration (1). When calculated in this manner, estrone and estriol markedly reduced the concentration of ^{3}H-estradiol in the hypothalamus so that the reduction was comparable to that found in the anterior pituitary, uterus and vagina. In contrast to estrone and estriol, even high doses of progesterone, testosterone and hydrocortisone did not reduce the concentration of ^{3}H-estradiol in tissues. With progesterone, there was a slight increase in the cerebral ^{3}H-estradiol concentration.

TABLE 1. Distribution of ^{3}H-estradiol in ovariectomized and intact female rats

	Intact $\mu\mu$c/mg ±SEM	Ovariectomized $\mu\mu$c/mg ±SEM
Anterior pituitary	260 ±20	344 ±60
Uterus	203 ±30*	290 ±15
Vagina	138 ±9.2	122 ±14
Adrenal	96 ±5.4	96 ±8.4
Fat	75 ±6.3	105 ±24
Muscle	39 ±16	22 ±2.8
Lung	28 ±5.4	36 ±6.3
Plasma	8.1 ±2.8	10 ±3.0
Preoptic region	21 ±2.3	18 ±2.0
Hypothalamus	21 ±2.8	22 ±3.6
Septum	16 ±3.6	23 ±2.0
Cerebrum	11 ±1.1	14 ±2.0
Cerebellum	9.9 ±2.7	11 ±1.7

^{3}H-estradiol, 0.1 μg/100 g, was injected intravenously. Tissue concentrations of ^{3}H-estradiol were measured 1 hr later.
* $p < .05$.

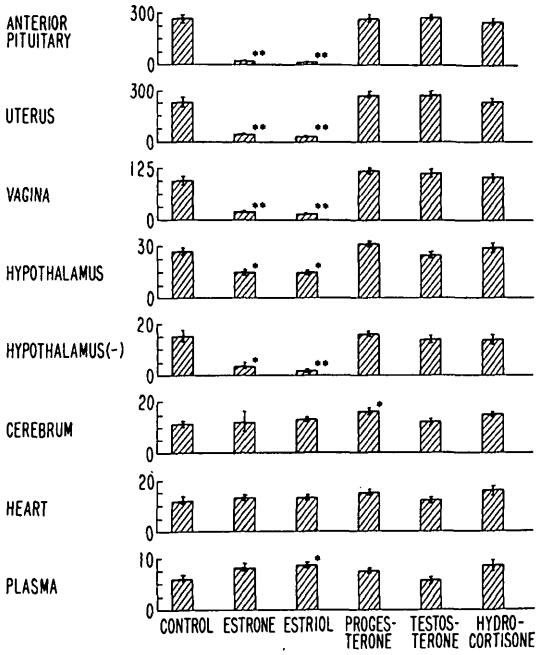

FIG. 1. Effect of steroid hormones on distribution of ^{3}H-estradiol. ^{3}H-estradiol, 0.1 μg/100 g, was injected iv 30 sec after 25 μg/100 g of estrone or estriol or 1 mg/100 g of progesterone, testosterone or hydrocortisone. Subtraction of the cerebral concentration from the hypothalamic concentration is expressed by the symbol (−). Results are expressed as $\mu\mu$c/mg ± the standard error of the mean. *$p < .05$, **$p < .001$ compared to control concentrations.

The distribution of estradiol was compared in mature ovariectomized and intact rats one hour after the administration of 0.1 μg/100 g ^{3}H-estradiol (Table 1). In terms of tissue concentration of ^{3}H-estradiol, the only significant difference found was a lower concentration in the uterus of the intact rat. However, the uterus of the intact rat weighed about four times as much as that of the ovariectomized rat so that the total content of ^{3}H-estradiol was about three times greater than that in the uterus of the ovariectomized rats. This suggested that an ovarian factor was increasing the total number of uterine binding sites for estradiol.

To examine the possibility that estradiol caused an increase in its own uterine binding capacity, ovariectomized rats were treated with estradiol for three days. When

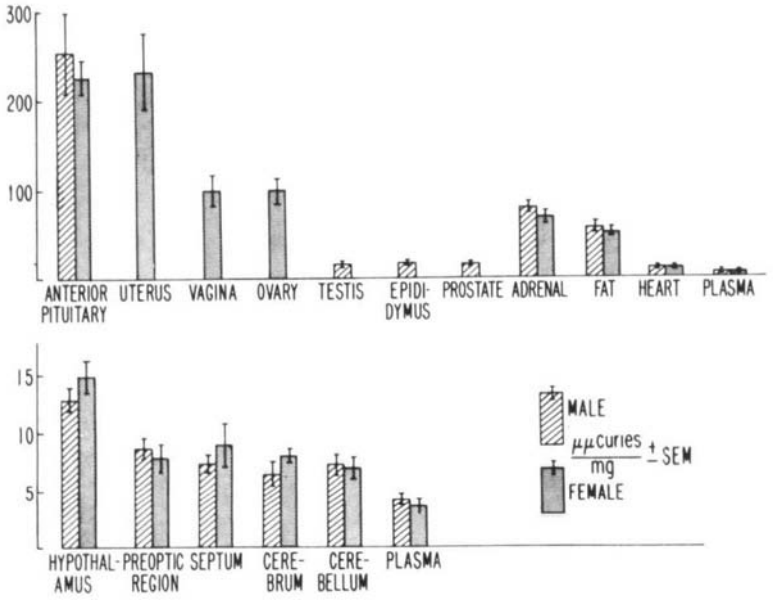

FIG. 2. Comparison of distribution of ^{3}H-estradiol in male and female rats. The distribution of 0.1 μg/100 g iv ^{3}H-estradiol was compared in mature intact male and mature intact female rats 1 hr after administration. Results are expressed as μμc/mg ±SEM.

a high dose of ^{3}H-estradiol was administered two days later, the uterus of the estrogen-primed rats contained about twice as much ^{3}H-estradiol and weighed twice as much as the uteri of untreated rats (Table 2). The vaginal weight also increased with estrogen priming, but the binding capacity for ^{3}H-estradiol did not change with estrogen pretreatment. The concentrations of ^{3}H-estradiol decreased in the anterior pituitary, uterus and vagina.

There were no differences in ^{3}H-estradiol accumulations in peripheral tissues of mature and immature female rats (Table 3). In the central nervous system the concentration of ^{3}H-estradiol was lower in most of the regions of the immature brain; however, plasma concentrations were also lower in the immature rat. When the ^{3}H-estradiol concentrations were expressed in terms of brain concentration to plasma concentration, or by subtracting the presumed nonspecific absorption component (1), there were no significant differences.

^{3}H-estradiol concentrations in the male anterior pituitary, hypothalamus, preoptic

TABLE 2. Effect of estrogen pretreatment on the distribution of ^{3}H-estradiol

	Estrogen-primed μμc/mg ±SEM	Control μμc/mg ±SEM
Anterior pituitary	1,100 ±60*	1,400 ±81
Uterus	1,500 ±160*	2,200 ±200
Vagina	700 ±63*	1,200 ±130
Hypothalamus	340 ±28	400 ±46
Hypothalamus (−)	77 ±18	81 ±33
Cerebrum	260 ±18	320 ±23
Heart	270 ±24	330 ±14
Plasma	190 ±29	217 ±20
Uterine weight (mg)	248 ±29**	107 ±11
Uterine ^{3}H-content (μμc)	350,000 ±14,000**	220,000 ±18,000
Vaginal weight (mg)	173 ±3.9**	114 ±6.2
Vaginal ^{3}H-content (μμc)	120,000 ±9,800	140,000 ±13,000

Ovariectomized rats were treated with 2 μg/100 g nonradioactive estradiol subcutaneously in saline daily for 3 days. Forty-eight hr later 2 μg/100 g ^{3}H-estradiol was injected intravenously, and tissue ^{3}H-estradiol concentrations were measured 1 hr later.

* $p < .05$; ** $p < .001$.

TABLE 3. Comparison of the distribution of ^{3}H-estradiol in immature and mature female rats

	Immature $\mu\mu$c/mg ±SEM	Mature $\mu\mu$c/mg ±SEM
Anterior pituitary	290 ±108	231 ±18
Uterus	342 ± 33	238 ±45
Vagina	140 ± 13	101 ±17
Ovary	108 ± 11	101 ±16
Adrenal	45 ± 5.9	66 ± 7.7
Fat	42 ± 5.2	49 ± 5.6
Heart	7.0 ± 0.66	8.0 ± 0.73
Plasma	2.3 ± 0.24	3.5 ± 0.73
Hypothalamus	10 ± 1.5*	15 ± 1.4
Septum	5.9 ± 0.66	9.1 ± 1.9
Preoptic region	4.0 ± 0.31*	8.3 ± 1.2
Cerebrum	4.4 ± 0.52*	8.0 ± 0.63
Cerebellum	4.0 ± 0.38*	7.0 ± 0.91
Ratio to plasma	$\frac{\mu\mu c/mg \text{ brain region}}{\mu\mu c/mg \text{ plasma}}$ ±SEM	$\frac{\mu\mu c/mg \text{ brain region}}{\mu\mu c/mg \text{ plasma}}$ ±SEM
Hypothalamus	4.3 ± 0.63	4.3 ± 0.60
Septum	2.5 ± 0.38	2.6 ± 0.61
Preoptic region	1.7 ± 0.21	2.4 ± 0.42
Cerebrum	1.9 ± 0.30	2.3 ± 0.30
Cerebellum	1.7 ± 0.23	2.0 ± 0.33
	$\mu\mu$c/mg ±SEM	$\mu\mu$c/mg ±SEM
Hypothalamus (−)	5.9 ± 0.77	7.7 ± 3.0
Hypothalamus (— —)	6.3 ± 1.2	8.0 ± 0.77
Septum (−)	1.5 ± 0.66	1.2 ± 1.6
Septum (− −)	2.0 ± 0.87	2.0 ± 1.4

^{3}H-estradiol, 0.1 μg/100 g, was administered intravenously to immature intact and mature intact female rats. Tissue ^{3}H-estradiol concentrations were measured after 1 hr. The brain region concentrations are also expressed as the ratio to the plasma concentrations and by subtraction of cerebral (−) or cerebellar (− −) concentration.

* $p < .05$ compared to concentrations in the mature female.

region and plasma were similar to those of the female (Fig. 2). Unlike the female sex organs, the testis, epididymis and prostate did not concentrate estradiol.

Discussion

Previous reports have indicated specific binding sites for estradiol in the anterior pituitary, uterus, vagina and hypothalamus. This conclusion has been based on the observation of higher accumulation and retention of the radioactive steroid in these organs (1, 2), saturation with low doses of estradiol (1) and competition for binding by compounds of related structure (1–3, 5).

This report has presented further evidence for the specificity of the estradiol binding sites in these organs. Administration of estrone and estriol reduced the accumulation of ^{3}H-estradiol in the anterior pituitary, uterus, vagina and hypothalamus, but not in organs such as the heart, cerebrum or plasma.

The effect of estrone or estriol on the accumulation in the hypothalamus was more marked when the difference between hypothalamic and cerebral concentration was compared in control and drug-treated animals. The basis for suggesting that analysis by difference should be considered is derived from three sources. First, radioautography has shown specific neurons which highly concentrate an estrogenic substance in only the hypothalamus and limbic system (4). Second, preliminary computer numerical analysis of the kinetics of distribution of ^{3}H-estradiol has been consistent with the hypothalamus containing two pools, with the "labile" pool similar to the total cerebral concentration (Eisenfeld, A. J., unpublished observations). Third, when the difference between hypothalamic

and cerebral concentration was considered, low doses of estradiol saturated the binding sites and norethynodrel functioned as a competitive inhibitor of estradiol accumulation (1).

The apparent competition by estrone for binding may be due to rapid conversion to nonradioactive estradiol, as has been previously suggested in studies of vaginal and uterine binding (2, 5). The binding sites for estradiol appear to be highly specific since other steroids such as progesterone, testosterone and hydrocortisone, even in high doses, did not reduce the binding of ^{3}H-estradiol. This is consistent with the recent report that testosterone or hydrocortisone did not change the accumulation of ^{3}H-estradiol in the uterus (6). These hormones are known to modify the effects of estradiol (7–9); this interaction cannot be due to these steroids attaching to the estradiol binding sites.

In comparing intact and ovariectomized rats, only accumulation in the uterus was different. The concentration of ^{3}H-estradiol in the uterus of rats with ovaries was less, but the intact uterus, which weighed more, contained three times as much ^{3}H-estradiol. In confirmation of a previous report (2), pretreatment of ovariectomized rats with estradiol not only caused an increase in weight but also caused an increase in the binding sites for the hormone in the uterus. The decrease in concentration of ^{3}H-estradiol in the anterior pituitary, uterus and vagina of the estrogen-primed rats might have been due to the persistence of the previously administered estrogen.

No difference was found in the distribution of ^{3}H-estradiol in peripheral tissues of immature and mature female rats. When the ^{3}H-estradiol concentrations in the brain regions were calculated as the ratio to plasma or by subtracting the presumed nonspecific absorption component, the pattern of distribution was similar in the immature and mature animals. Thus, it would appear that the physiologic changes of puberty are not controlled by changes of the estradiol binding sites.

In contrast to the female sexual organs, the testis, prostate and epididymis did not concentrate ^{3}H-estradiol. This is consistent with previous findings that estradiol did not accumulate in the prostate or seminal vesicle (10). The distribution of ^{3}H-estradiol in the anterior pituitary, hypothalamus, preoptic region and septum of male and female rats was similar. Thus, differences in the pattern of gonadotrophin secretion cannot be explained on the basis of differences of the estradiol binding sites in these regions.

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