*ESTROGEN "RECEPTORS" IN BRAIN: AN UNSOLVED PROBLEM**

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Abstract.—Various investigators have postulated that estrogen-sensitive structures contain "receptors" which accumulate and bind estradiol. Further, it has been implied that brain receptors function in a similar way to uterine receptors, even though the evidence for this conclusion is not strong. The present study was designed to compare the accumulation of radioactivity by brain and uterus following ^{3}H-estradiol treatment in estrous, diestrous, and ovariectomized rats. Uterus exhibited high levels of radioactivity in diestrus, but not in estrus or the ovariectomized condition. The accumulation of hormone by brain did not fluctuate with the various levels of endogenous hormone stimulation, but rather showed a pattern of uptake similar to muscle, a nontarget tissue. It was concluded that if brain hormone receptors exist, they do not respond to estradiol as do uterine receptors.

Several studies have demonstrated that the uterus has a great affinity for estradiol and is capable of retaining that hormone for many hours.[1–5] This observation has led to the hypothesis that the uterus contains "receptors" which specifically accumulate and bind estradiol.[6, 7] In the past few years investigators have also suggested that estradiol receptors exist in the brain as well as the uterus and that these brain hormone receptors have characteristics similar to those found in the uterus.[8, 9] The evidence for such a conclusion, however, is not strong. Autoradiographic evidence does indicate that estradiol is accumulated and retained by brain.[10–12] However, these autoradiographic studies do not present a comparison of the pattern of uptake and retention of hormone by brain and uterus and so provide no evidence on the similarity of these hormone-sensitive systems.

Two studies employing the liquid scintillation technique do present comparisons between brain and uterus which indicate that these structures respond differently to estradiol.[3, 5] Both show that after the administration of tritiated estradiol, radioactivity increases or remains stable for at least one hour in uterus, but declines rapidly in brain during that period.

The purpose of the present study was to compare the response to estradiol of hormone-sensitive tissues (uterus, pituitary, and brain) with the response of nonhormone-sensitive tissue (muscle) under various conditions of endogenous hormone secretion. It was felt that similar patterns of reactivity to estradiol would provide additional evidence for the similarity of brain and peripheral hormone receptor systems.

Materials and Methods.—Tritiated estradiol was administered to gonadally intact female rats (N = 13) at various stages of the estrous cycle and to females which had been ovariectomized for 2 weeks prior to treatment (N = 5). The rats, all of the Sprague-Dawley strain, weighed 283 gm on the average at the time of treatment. The gonadally intact animals were divided into three groups on the basis of their vaginal smear pattern.

Vaginal smears were taken daily for at least 8 days to ensure that the animals were displaying normal cycles. The average cycle length was found to be 4.4 days. Of the total smears taken, 26.9% were classified estrus, 48.1% diestrus, 7.7% metestrus, and 17.3% proestrus. Vaginal smears taken less than 2 hr before treatment were used to subdivide the cyclic females into three groups: (*a*) estrus—cornified cells ($N = 4$); (*b*) proestrus—nucleated cells ($N = 4$); or (*c*) diestrus—leukocytic smear pattern ($N = 5$).

At the time of treatment all rats were injected via the external jugular vein while under light ether anesthesia with 0.226 μg of 6,7 ^{3}H-estradiol 17-β (S.A. = 48.0 mc/μM, New England Nuclear) in 0.2 ml of 10% ethanol and were decapitated 30 min later.

The ventral body wall of each animal was then opened, and a muscle sample taken. The first 2 cm beyond the bifurcation of one uterine horn was removed.

The skull was opened and the brain removed and immediately sectioned as follows: The brain was placed on its dorsal surface and two transverse cuts were made, the first just caudal to the mammillary bodies and the second at the level of the optic chiasma approximately 5 mm anterior to the first cut. Sagittal cuts were then made 2 mm on each side of the midline. Finally, one horizontal cut was made 2 mm dorsal to the ventral surface of the brain. The section (average weight = 120.3 mg) is here referred to as the hypothalamic sample.

After removal of the brain from the skull case, the entire hypophysis was removed from the sella tursica and is here referred to as the pituitary sample.

All samples were placed in preweighed scintillation vials and reweighed; 1 ml of NCS tissue solvent was added to each vial, and the vials were transferred to a 37°C room. Following tissue dissolution, 15 ml of scintillation fluor were added to each vial. The fluor consisted of PPO (2,5-diphenyloxazole) and POPOP (1,4-bis(2-(4-methyl-5-phenyl-oxazolyl))) dissolved in analytical-grade toluene at concentrations of 5.0 and 0.05 gm/liter, respectively.

Samples were counted on a Beckman LS-150 scintillation counter. The counter read the full tritium range and accumulated sufficient counts to achieve a 95% confidence interval $\pm 2\%$ of the mean. The argon flush standard achieved approximately 57% efficiency while our samples ranged from 31.7 to 36.3% ($\bar{X} = 33.9\%$) efficiency as determined by an external standard channels-ratio technique. All samples were corrected for quenching, and data are reported as dpm. Data were subjected to analyses of variance.

Results.—The retention of radioactivity 30 minutes after estradiol treatment is indicated in Figures 1 and 2. Statistical analyses are presented in Table 1. Hypothalamic, pituitary, and muscle samples showed similar patterns under the

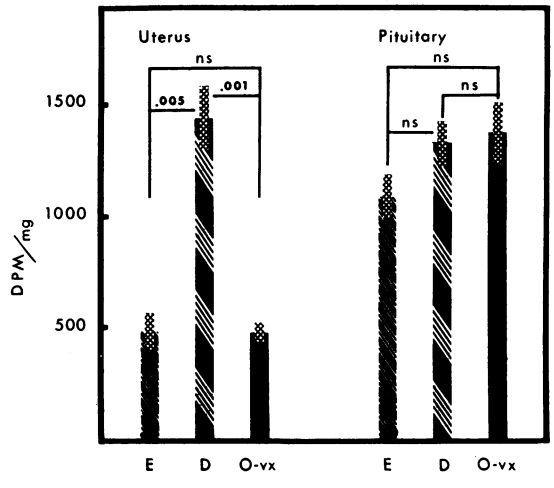

FIG. 1.—Radioactivity level in uterus and pituitary of estrous (*E*), diestrous (*D*), and ovariectomized (*O-vx*) rats 30 min after treatment with tritiated estradiol.

TABLE 1. *Statistical comparisons of radioactivity levels in various tissues of estrous, diestrous, and ovariectomized rats after treatment with tritiated estradiol.*

Tissue	Condition	*F*	*df*	*p*
Uterus	Estrus vs. diestrus	22.9	1, 7	<0.005
	Diestrus vs. O-vx	31.6	1, 8	<0.001
	Estrus vs. O-vx	1.0	1, 7	NS*
Hypothalamus	Estrus vs. diestrus	4.0	1, 6	NS
	Diestrus vs. O-vx	8.2	1, 7	<0.05
	Estrus vs. O-vx	1.0	1, 6	NS
Pituitary	Estrus vs. diestrus	1.9	1, 7	NS
	Diestrus vs. O-vx	1.0	1, 8	NS
	Estrus vs. O-vx	2.0	1, 7	NS
Muscle	Estrus vs. diestrus	1.1	1, 7	NS
	Diestrus vs. O-vx	4.3	1, 8	NS
	Estrus vs. O-vx	1.5	1, 7	NS

* NS, not significant.

three conditions of endogenous hormone, patterns which were strikingly different from that of uterus.

Estrus vs. diestrus: The radioactivity level was approximately three times as high in the uterus from diestrous rats as from estrous rats. This estrus-diestrus difference was not found in hypothalamus, muscle, or pituitary samples.

Diestrus vs. ovariectomized condition: The radioactivity level was approximately three times as high in the uterus from diestrous rats as from ovariectomized rats. No significant differences were found under these conditions for muscle or pituitary samples. For hypothalamic samples the radioactivity level was significantly lower in diestrous than ovariectomized animals, 92.4 dpm/mg vs. 116.7 dpm/mg.

Estrus vs. ovariectomized condition: The radioactivity levels for uterus were similar in estrous and ovariectomized rats, 483.0 dpm/mg and 483.1 dpm/mg, respectively. A similar absence of significant differences between these conditions were found in hypothalamic, muscle, and pituitary samples.

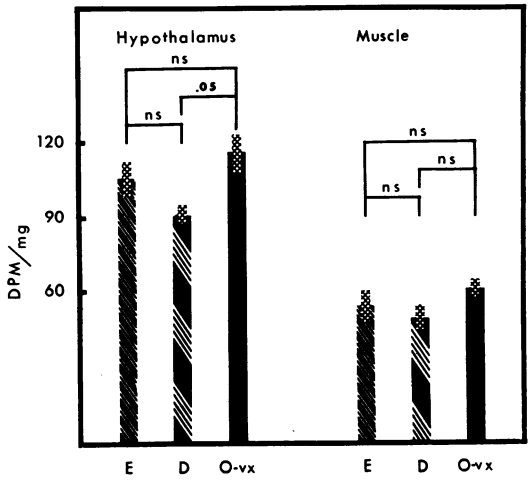

FIG. 2.—Radioactivity level in hypothalamus and muscle of estrous (*E*), diestrous (*D*), and ovariectomized (*O-vx*) rats 30 min after treatment with tritiated estradiol.

Proestrus: For all samples radioactivity levels were similar in proestrous and estrous conditions. Proestrous levels were as follows: uterus—550.0 dpm/mg; pituitary—937.0 dpm/mg; Hypothalamus—89.6 dpm/mg; muscle—49.2 dpm/mg.

Discussion.—The present findings indicate that the accumulation of exogenous estradiol by the uterus of gonadally intact rats is inversely related to presumed levels of circulating hormone. Uterine radioactivity levels were found to be high during diestrus and low during estrus. This observation and similar data reported by McGuire and Lisk[4] parallel earlier findings[13] that "cold" estradiol can competitively inhibit the accumulation of radiolabeled hormone by the uterus. These various observations are all consistent with the postulated characteristics of uterine estradiol receptors.

In contrast, the present study failed to reveal significant variations in radioactivity level as a function of the estrous cycle in brain, muscle, or pituitary. The fact that hypothalamic tissue responded to estradiol much in the manner of muscle, which is not normally considered a hormone-sensitive tissue, raises the question of whether or not brain hormone receptors, if such exist, do possess functional characteristics similar to those of uterine receptors. The present data indicate that they do not. These data and this interpretation are not in agreement with the findings of McGuire and Lisk, who reported great similarity in the response of brain and uterus to estradiol. The source of our differences is unclear. However, the two studies did differ in hormone dose and time of sacrifice.

A second finding from the present study also suggests essential differences between uterus and other hormone-sensitive tissue. Following ovariectomy and the reduction of circulating estrogen, the uterus loses its ability to accumulate estradiol. As shown in Figure 1, uterine radioactivity levels were low in the castrate "diestrous" animal in comparison with the intact diestrous animal, indicating that the reduction in endogenous estrogen of the cycling animal at diestrus has a profoundly different effect upon the accumulation of estradiol than does the elimination of endogenous hormone by castration. This phenomenon, together with the observation that estradiol priming of the castrate female rodent can result in a facilitation of hormone uptake by uterus,[2] has led to the hypothesis that the functional integrity of uterine estradiol receptors is dependent upon a low continuous level of hormone stimulation. In fact, it has been suggested that following ovariectomy, uterine estradiol receptors degenerate in the absence of hormone stimulation.[4] The brain does not seem to display this characteristic of the uterus. As shown in Figure 2, hypothalamic radioactivity levels in the animals which had been ovariectomized for two weeks were not significantly different from the levels in intact estrous animals and were in fact slightly higher than the levels found in intact diestrous animals. Thus, the variations in the accumulation of hormone by the uterus which systematically follow endogenous hormone stimulation are not found in brain.

The present study should not be taken to indicate that estrogen receptors do not exist in brain. Our findings simply raise questions about the functional characteristics of the neural response to hormone. These data and earlier find-

ings from our laboratory on the duration of retention of estrogen in brain and uterus[5] lead us to believe that the uterus does not provide an adequate model for understanding the effects of hormone on brain.

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