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EVIDENCE FOR ESTRADIOL BINDING SITES IN THE HYPOTHALAMUS—EFFECT OF DRUGS

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Abstract—One hr after i.v. injection, the concentration of ^{3}H-estradiol in hypothalamus, anterior pituitary, uterus, and vagina of ovariectomized rats was lowered by prior administration of diethylstilbestrol, clomiphene, dimethylstilbestrol, U11100, or norethindrone. The concentration of ^{3}H-estradiol in heart, cerebrum or plasma was little, if any, reduced by these drugs. Chlormadinone and medroxyprogesterone did not decrease the concentration of ^{3}H-estradiol in any organ. These data support the thesis that binding sites for estradiol are present in the hypothalamus and that interaction by specific drugs with the estradiol-binding site in the hypothalamus may be a mechanism for regulation of ovulation.

PREVIOUSLY, it has been shown that certain drugs reduce the accumulation of radioactive estradiol in target organs, presumably by competition for combination with a limited number of macromolecules in these organs.[1–7] In this report the effect of i.v. administration of several drugs, most of which are known to influence ovulation, on the concentration of ^{3}H-17β-estradiol in the hypothalamus in addition to that in the anterior pituitary, uterus, vagina, heart, cerebrum, and plasma will be described. The drugs studied were: clomiphene, which promotes ovulation in women with secondary amenorrhea; three progestins, norethindrone, chlormadinone, and medroxyprogesterone; dimethylstilbestrol and U11100, which can reduce some estrogenic effects of co-administered estradiol; and diethylstilbestrol.

MATERIALS AND METHODS

The distribution of ^{3}H-estradiol was studied in mature Sprague–Dawley rats, ovariectomized at least 2 weeks prior to experimentation. Six animals were used in each control and drug-treated group. The compounds used were: diethylstilbestrol, 3,4-*bis*(*p*-hydroxyphenyl)-3-hexene, 25 μg/100 g; clomiphene, 1-(*p*-(β-diethylaminoethoxy)phenyl)-1-2-diphenyl-2-chloroethylene citrate, 5 mg/100 g; chlormadinone, 6-dehydro-6-chloro-17-α-acetoxyprogesterone, 1mg/100 g; medroxyprogesterone acetate, 6α-methyl-17α-acetoxyprogestrone, 1mg/100 g; dimethylstilbestrol, 2,3-*bis*(*p*-hydroxyphenyl)-2-butene, 100 μg/100 g; U11100, 1-(2-(*p*-(3,4 dihydro-6-methoxy-2-phenyl-1-napthyl)phenoxy)ethyl)-pyrrolidine hydrochloride, 1 mg/100 g; norethindrone, 17α-ethinyl-estr-4-en-17β-ol-3-one, 800 μg/100 g. The doses were selected on the basis of preliminary studies to obtain a pronounced effect and the drugs were administered shortly before the estradiol to minimize competition by their

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metabolites for binding. The drugs were dissolved in 0·2 cc absolute ethanol and were injected i.v.; the controls received the ethanol alone. ^{3}H-estradiol (9·7 c/m-mole, New England Nuclear Corp.), 0·1 μg/100 g body wt., was administered in aqueous solution by tail vein 30 sec later. One hr after injection, organs were removed, weighed, and frozen until analyzed. As previously described,[4, 7] the organs were homogenized in 2 ml water and extracted with 10 ml 95% toluene: 5% isoamyl alcohol. The organic phase was counted for radioactivity. In control animals about 90 per cent of the radioactivity extracted is estradiol except for that from plasma. Plasma radioactivity consists of 50 per cent estradiol and 40 per cent estriol; evidence has previously been presented that estriol can also occupy binding sites.[7]

RESULTS

In accord with previous reports[4, 8–10] the concentration of ^{3}H-estradiol 1 hr after i.v. administration was relatively high in the anterior pituitary, uterus, and vagina; the

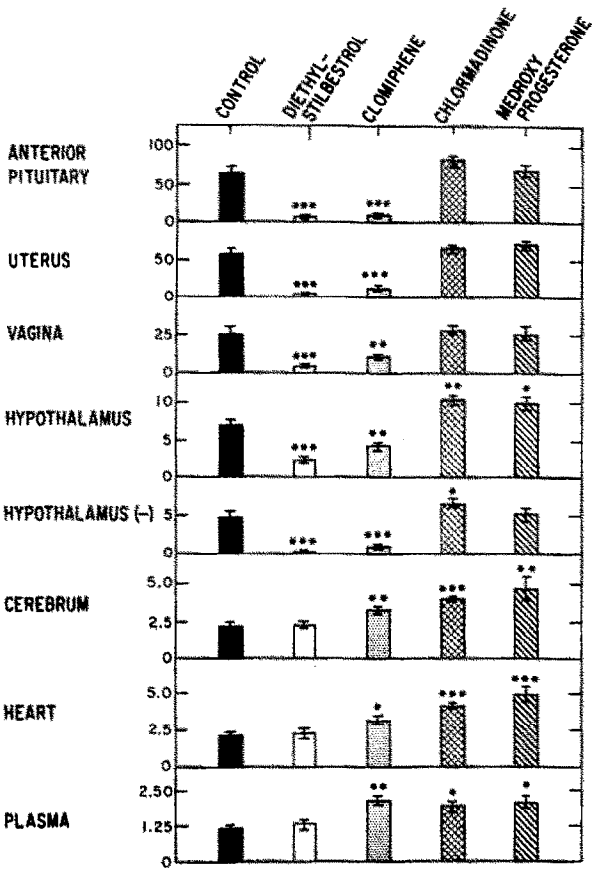

FIG. 1. Effect of drugs on the distribution of ^{3}H-estradiol. Diethylstilbestrol (25 μg/100 g), clomiphene (5 mg/100 g), chlormadinone (1 mg/100 g) or medroxyprogesterone (1 mg/100 g) was injected i.v. 30 sec before 0·1 μg ^{3}H-estradiol/100 g. Tissues were analyzed for ^{3}H-estradiol concentration 1 hr later. Results are expressed as μμc/mg ± S.E.M. Hypothalamus (–) concentrations represent the difference between hypothalamic and cerebral concentrations in each animal. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ compared to concentrations in the controls.

concentration of ^{3}H-estradiol in the hypothalamus was twice that in the cerebum (Figs. 1 and 2). Pretreatment with diethylstilbestrol, clomiphene (Fig. 1), dimethylstilbestrol, U11100 or norethindrone (Fig. 2) lowered the accumulation of ^{3}H-estradiol in the anterior pituitary, uterus, vagina, and hypothalamus.

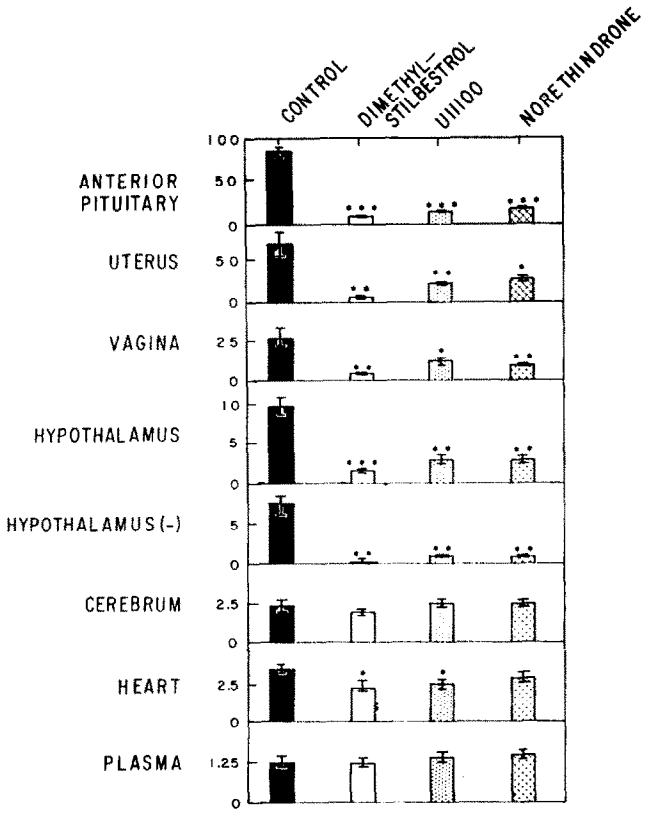

FIG. 2. Distribution of ^{3}H-estradiol after administration of dimethylstilbestrol, U11100 or norethindrone. Dimethylstilbestrol (100 μg/100 g), U11100 (1 mg/100 g) or norethindrone (800 μg/100 g) was administered by tail vein 30 sec before 0·1 μg ^{3}H-estradiol/100 g. The concentration of ^{3}H-estradiol in tissues 1 hr later is indicated as $\mu\mu$c/mg $\pm$ S.E.M. (*) $P < 0{\cdot}05$, (**) $P < 0{\cdot}01$, (***) $P < 0{\cdot}001$ compared to concentrations in the controls.

The concentration of estradiol in the hypothalamus was not reduced by these drugs as markedly as in the anterior pituitary, uterus, and vagina. However, it has been suggested that ^{3}H-estradiol may be present in the hypothalamus attached to a small number of specific binding sites and non-specifically, possibly in free form.[4] Based on digital computer analysis of the kinetics of distribution after i.v. administration, the cerebrum appears to consist only of the 'free' component and to be similar in magnitude to the 'free' component in the hypothalamus.[11] Thus, the specific hypothalamic component may be estimated by the difference between the hypothalamic and cerebral concentration of ^{3}H-estradiol in each animal. When calculated in this manner, the reduction of ^{3}H-estradiol accumulation in the hypothalamus by administration of the stilbestrols, clomiphene, U11100, and norethindrone, was more pronounced and was comparable to the reduction in the anterior pituitary, uterus, and vagina.

Both diethyl- and dimethylstilbestrol almost completely abolished target organ binding of estradiol; in contrast, even high doses of clomiphene, U11100 or norethindrone only partially reduced the levels of radioactivity. Two progesterone derivatives, chlormadinone and medroxyprogesterone, at doses of 1 mg/100 g, did not reduce the accumulation of ^{3}H-estradiol.

The concentration of ^{3}H-estradiol in heart, cerebrum, and plasma was relatively low in the controls and with administration of these drugs there was no substantial reduction in the concentration of ^{3}H-estradiol in these organs. No explanation is apparent for the slight reduction in the ^{3}H-estradiol concentration in the heart after dimethylstilbestrol or U11100. The elevation of the concentration of ^{3}H-estradiol in heart and cerebrum and plasma found with clomiphene, chlormadinone, and medroxyprogesterone may be due to competition for metabolism or clearance from plasma between estradiol and high dose of these drugs.

DISCUSSION

By measuring the concentration of ^{3}H-estradiol, the presence of binding sites for estradiol in target organs has been suggested on the basis of high accumulation,[4, 8–10] dose-related reduction of ^{3}H-estradiol levels with administration of specific drugs,[1–7] and limited estradiol accumulation with increasing doses administered.[4–6] A recent abstract has suggested that the binding sites for estradiol in the anterior pituitary, uterus, and vagina may be nuclear protein.[12] (The uterus also appears to have protein binding sites for estradiol in the cytoplasm.[13])

Decreased accumulation of ^{3}H-estradiol has previously been reported with administration of diethylstilbestrol (nuclear fraction of the uterus),[5] clomiphene (anterior pituitary and uterus),[2] dimethylstilbestrol (uterus),[3] and U11100 (anterior pituitary, uterus, and vagina).[1] This report indicates that, in addition to lowering the concentration of ^{3}H-estradiol in the anterior pituitary, uterus, and vagina, the concentration of estradiol in the hypothalamus is also decreased in the presence of diethylstilbestrol clomiphene, dimethylstilbestrol, U11100 or norethindrone.

Prior evidence from measurement of ^{3}H-estradiol concentration in the hypothalamus for specific binding sites has been: (a) higher retention than in brain regions such as cerebrum;[4] (b) reduced ^{3}H-estradiol levels with administration of estriol or norethynodrel;[4, 7] and (c) saturation with increasing doses administered when the 'bound' component was estimated by the difference between hypothalamic and cerebral concentration,[4] as suggested by digital computer analysis of the kinetics of distribution.

From another approach, by radioautographic techniques, an estrogenic substance, ^{3}H-hexestrol, was found to localize in the hypothalamus, possibly concentrated in certain neurons.[14] The biochemical nature of the estradiol-binding site in the hypothalamus remains to be determined.

Administration of the compounds that reduce target organs binding of estradiol can produce some effects similar to those of estradiol (diethylstilbestrol, clomiphene,[15] dimethylstilbestrol,[16] U11100[17] or norethindrone[16]). Alternatively these drugs can reduce some of the effects of co-administered estradiol (clomiphene,[15] dimethylstilbestrol,[16] U11100[17] or norethindrone[16]). Although it has not been established, it is possible that these compounds may reproduce the effects of estradiol to some extent because of their interaction with the binding sites for estradiol or they may reduce

the effects of co-administered estradiol by decreasing the availability of binding sites for occupation by this estrogen with high intrinsic activity.

Increased secretion of estradiol by the ovary at the midportion of the menstrual cycle is thought to trigger ovulation,[18] and clomiphene administration has produced ovulation in anovulatory women.[19] Alternatively, it has been shown that ovulation is inhibited or gonadotrophin secretion is decreased, or both, by administration of estradiol,[20] diethylstilbestrol[21] or norethindrone[22] to women or clomiphene to animals.[23] The regulation of ovulation by drugs is generally thought to occur at the hypothalamic level.[18] The presence of binding sites in the hypothalamus for estradiol is consistent with this concept. These drugs may influence ovulation by their interaction with the hypothalamic estradiol-binding site.

The hypothalamus may not be the only region in the central nervous system for regulation by estradiol of hypothalamic gondotrophin-releasing factors and pituitary gonadotrophins. Binding of estradiol in the anterior pituitary or the preoptic region and septum of the limbic system[4, 14] may also be involved.

Ovulation may also be regulated by another central nervous system mechanism, sensitive to drugs with progestational activity,[18] which does not appear to involve the estradiol-binding sites. This second mechanism may be responsible for inhibition of ovulation by progesterone,[24] chlormadinone[25] or medroxyprogesterone[26] and possibly norethindrone.[22]

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