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The Uptake of Progesterone by the Brain Tissues of Male and Female Rats *in vivo* and Its Relationship to the Cytoplasmic Progesterone-binding Component

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The uptake of ^{3}H-progesterone by the brain tissues of gonadectomized male and female rats following an i.v. injection of radiolabeled hormone was studied. In females, ^{3}H-progesterone concentration was highest in the median eminence, followed by the posterior through anterior hypothalamus to the cerebral cortex. In addition, the median eminence/cortex ratio of radioactivity increased almost linearly with time after the injection. In males, radioactive progesterone concentration in all the hypothalamic regions exceeded that in the cerebral cortex. Among the four basal brain tissues, the median eminence showed the highest tissue/cortex ratio of radioactivity.

The progesterone-binding activity of the female rat hypothalamic 107,000 x *g* supernatant was examined *in vitro* by Sephadex G-200 column analysis. Protein components in the cytosol from the median eminence with high-affinity limited-capacity progesterone-binding activity were identified.

It appears that the preferential uptake of progesterone by the median eminence of the female rat hypothalamus is correlated with the protein components which have high-affinity limited-capacity progesterone-binding activity in the tissue.

(Key Words : Progesterone, Hypothalamus, Cytosol, Receptor Protein)

Recent knowledge of the mechanisms by which steroid hormones act on target tissues indicates that binding of hormone by specific cytoplasmic receptor proteins is an initial event. It is assumed that the binding of hormones by the receptor protein is followed by movement of the protein-hormone complex to the cell nucleus where alteration of the cellular function is initiated. This two step mechanism was put forth independently by Jensen et al. (7, 8) and Gorski et al. (4), suggesting that mechanism is characteristic of all sex steroids. O'Malley (13) has reported that this theory is generally applicable to steroid hormones.

Essential to this concept of specific steroid receptors are the observations that the hormone must be present in blood at sufficient concentrations to encounter sufficient receptor molecules in tissues and that target tissues selectively take up hormones from blood, whereas non-target

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tissues do not (5, 6). There have been several papers concerning the ability of brain tissues to take up progesterone selectively from blood *in vivo*. Seiki et al. (16, 17) injected ^{3}H-progesterone into ovariectomized rats and found an increasing gradient of radioactivity from the anterior to posterior hypothalamus, followed by the cerebral cortex. Luttge et al. (10) administered tritiated progesterone to ovariectomized mice and observed maximal accumulation of radioactive hormone in the interpeduncular region of the midbrain and to a lesser extent in the hypothalamus. Wade and Feder (23, 24) injected ^{3}H-progesterone into guinea pigs and found the highest uptake of the hormone in the midbrain, followed by the hypothalamus, cerebral cortex and hippocampus. Wade et al. (25) injected ^{3}H-progesterone into ovariectomized rats, guinea pigs and hamsters and found the highest uptake of the radioactive hormone in the midbrain of rats and guinea pigs, followed by the hypothalamus. They also found that the midbrain uptake was highest in the hamster brain, but no consistent uptake pattern was found in the other brain tissues. These findings strongly suggest that progesterone may play a role in neuroendocrine regulation by its action in the midbrain or in the medial basal hypothalamus in these species (14, 15).

Due to the importance of these findings in our understanding of the role and mechanism of progesterone action in the brain, it is considered worthwhile to examine more precisely the uptake of progesterone *in vivo* by the brain including the hypothalamus and its relationship to subcellular hormone-binding proteins.

In vivo uptake of progesterone by rat brain tissues

A) Females

Adult ovariectomized Wistar strain rats, weighing 200—220 g, were primed daily with estradiol benzoate (2 μg in sesame oil, s. c.) for 3 days. ^{3}H-progesterone (45 μc/100 g body weight in 10% ethanol-saline solution; sp. act. 750 mCi/mM, The Radiochemical Center, England) was then injected i. v. At regular intervals following the injection the animals were sacrificed by decapitation, and the hypothalamus and the cerebral cortex were excised. The median eminence was resected from the hypothalamus and the remainder of the brain was divided into the anterior, middle and posterior parts according to the method described by Kato and Villee (9). After homogenizing the tissue the homogenate was extracted with dichloromethane, and the extract was applied to a thin-layer plate using a solvent system of benzene-ethyl acetate (3 : 2). After visualizing the progesterone spot under a U-V lamp (254 mμ wave length), the spot was subjected to a radioactivity measurement.

As shown in Fig. 1, radioactivity in each tissue reached a maximum within 1/2 hr after ^{3}H-progesterone injection. At each time interval the highest uptake was found in the median eminence, followed by the posterior through the anterior hypothalamus to the cerebral cortex. Futhermore, the radioactivity in the median eminence was retained for 2 to 6 hr after the injection. Fig. 2 shows the tissue/cortex ratio of radioactivity concentration. The ratio in the median eminence increased

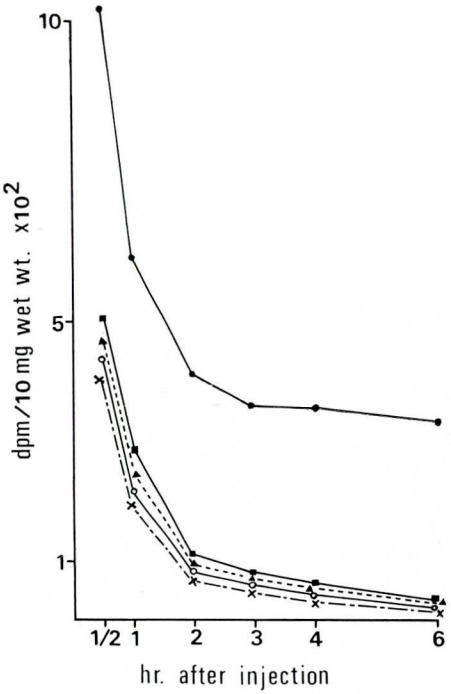

Fig. 1 Radioactivity pattern in various brain tissues of ovariectomized rats after an i. v. injection of ^{3}H-progesterone. Values are the average of 7 determinations. ●——● median eminence; ○——○ anterior hypothalamus; ▲·····▲ middle hypothalamus; ■——■ posterior hypothalamus; ×——× cerebral cortex.

almost linearly up to 6 hr, whereas the remaining hypothalamic regions showed no increase in the ratio with time.

These findings, are, in part, consistent with those reported by previous investigators (16—18, 26, 27) indicating the preferential uptake of progesterone by the female rat hypothalamus. Furthermore, the present results strongly suggest that the hypothalamus, particularily, the median eminence, may contain the binding sites for progesterone.

B) Males

Adult orchiectomized Wistar strain rats, 200—250 g, were injected intravenously with ^{3}H-progesterone (25 μc/100 g body weight in 10% ethanol-saline solution ; sp. act. 750 mCi/mM, The Radiochemical Center, England). At regular intervals following the injection, the animals were killed by decapitation and the hypothalamus and the cerebral cortex were excised. Further procedures for dissecting the brain tissues and analizing radioactivity in these tissues have already been described.

As shown in Fig. 3, radioactivity in each tissue was highest 20 min

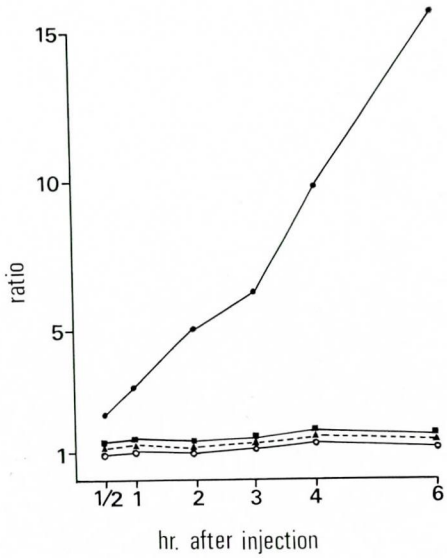

Fig. 2 Ratio of radioactivity concentration between hypothalamic tissue and cerebral cortex calculated from data in Fig. 1. Radioactivity in the cerebral cortex at each time is taken as 1. ●——● median eminence; ○——○ anterior hypothalamus; ▲·····▲ middle hypothalamus; ■——■ posterior hypothalamus.

after the injection, and gradually decreased in most tissues up to 160 min. Among the tissues, the median eminence took up more radioactivity at 80 and 160 min than did the other brain tissues, giving an elevated peak at 80 min. The four basal brain tissues showed elevated tissue/cortex ratios of radioactivity at each time point. Among these tissues the median eminence revealed an increasing tissue/cortex ratio with time, giving the highest ratio at 160 min.

These findings are consistent with those observed in female rats in the present study and with those reported by previous investigators (18, 26), indicating preferential uptake of progesterone even by the male rat hypothalamus. It is also suggested that the hypothalami of male and female rats do not differ in sensitivity to the hormone, but rather there are differences between males and females in molecular processes subsequent to the uptake of progesterone by the hypothalamic nuclei.

In vitro cytoplasmic progesterone-binding components in female rat hypothalamus

Adult ovariectomized rats pretreated with estradiol benzoate, as described previously, were killed by decapitation. The same hypothalamic regions as in the *in vivo* cases were dissected out and rinsed in cold buffer consisting of 10 mM Tris-HCl (pH 7.4) with 1 mM EDTA. Separate tissues from 20 to 30 rats were pooled and homogenized. The

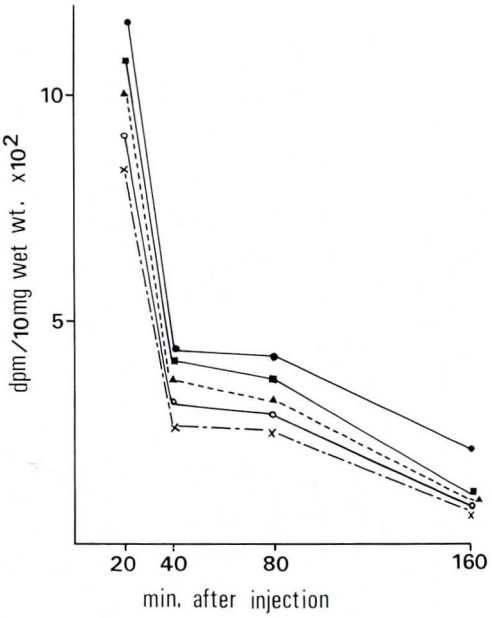

Fig. 3 Radioactivity pattern in various brain tissues of orchiectomized rats after an i. v. injection of ^{3}H-progesterone. Value is the average of 5 determinations. ●——● median eminence; ○——○ anterior hypothalamus; ▲·····▲ middle hypothalamus; ■——■ posterior hypothalamus; ×——× cerebral cortex.

supernatant fraction, the so-called "cytosol", was then obtained by centrifuging the homogenate at $800 \times g$ for 10 min, followed by $107,000 \times g$ for 1 hr. The cytosol was immediately used for incubation. In some cases it was diluted with the buffer to obtain the desired protein concentration. As indicated in the results, ^{3}H-progesterone (sp. act. 80Ci/mM, New England Nuclear, U. S. A.), ^{3}H-corticosterone (sp. act. 40Ci/mM, New England Nuclear, U. S. A.) and various unlabeled steroids were dried in a tube, and the cytosol was added to the tube. The tube was agitated in an ice-cold bath for the desired time.

A) Sephadex column chromatography

Each hypothalamic cytosol incubated with 10^{-9} M ^{3}H-progesterone was chromatographed on a Sephadex G-200 column at 4°C to separate bound from free radioactivity. A 0. 3 ml aliquot of the incubate was layered on the column and 1 ml fractions were collected. The fractions were then measured for radioactivity. Human γ-globulin and bovine serum albumin were subsequently chromatographed to determine appoximate molecular size of progesterone-binding proteins.

The Sephadex assay yielded two bound peaks in the median eminence cytosol (Fig. 4). The first major peak was of a macromolecule larger than the molecule of γ-globulin and the second minor peak B probably a smaller molecule than that of bovine serum albumin. Chro-

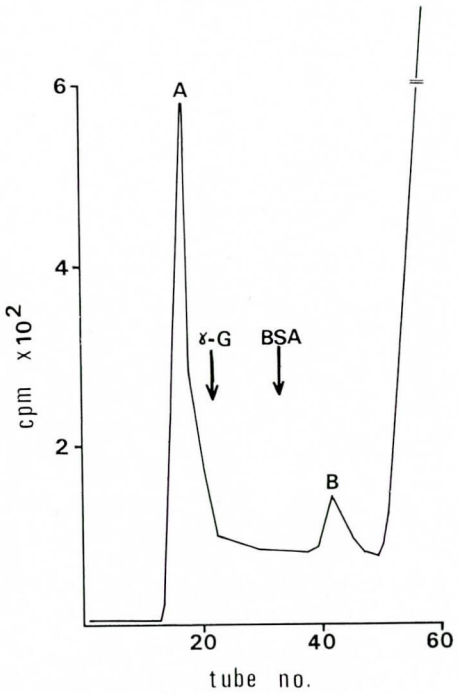

Fig. 4 Chromatography on Sephadex G-200 of $107,000 \times g$ cytosol from 20 ovariectomized rat median eminences in 0.3 ml of 10 mM Tris-HCl (pH 7.4) with 1 mM EDTA. Peaks A and B contain bound ^{3}H-progesterone. Column size: 80 cm × 0.9 cm. Elution volume: 1 ml/tube.

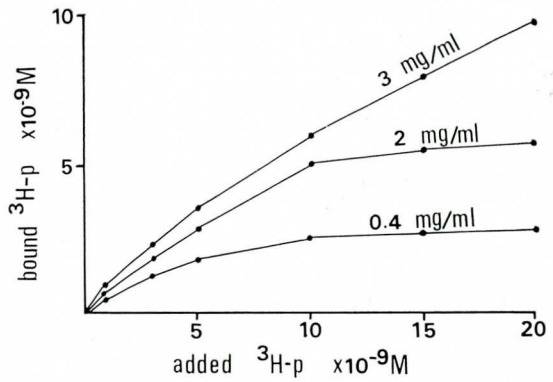

Fig. 5 Effect of dilution of median eminence cytosol from ovariectomized rats on tritiated progesterone binding to cytosol protein. Horizontal scale: total concentration of ^{3}H-progesterone added to the incubate; vertical scale: concentration of ^{3}H-progesterone bound to protein in the incubate.

Table 1. Effect of various unlabeled steroids on ^{3}H-progesterone binding to cytosol protein from ovariectomized rat median eminence. 10^{-9} M ^{3}H-progesterone was added to the cytosol.

Unlabeled steroids added to the cytosol		Radioactivity recovered in cytosol protein, cpm $\times 10^{3}$
None		422
Corticosterone	10^{-7} M	421
Estradiol-17β	10^{-7} M	416
Progesterone	10^{-7} M	4

matography of the remainder of the hypothalamus showed no conspicuous peaks bound to cytosolic proteins. All the results obtained here were reproducible over several repetitions.

B) Binding capacity

The capacity of the median eminence cytosol to bind progesterone was examined. At first, cytosol in different protein concentrations was incubated with 10^{-9}—2×10^{-8} M ^{3}H-progesterone and then passed through a millipore filter (pore size 0.45 μ). As shown in Fig. 5 no saturation of the cytosol protein was obtained at concentrations higher than 3 mg/ml, whereas saturation was obtained at concentrations lower than 2 mg/ml. Secondly, the cytosol with the 2 mg/ml protein concentration was incubated with 10^{-9} M labeled progesterone and 10^{-9}—10^{-7} M unlabeled progesterone. A slight replacement of labeled with unlabeled hormone was observed at 10^{-9} M, while almost complete replacement occurred at 10^{-8} M. The binding protein in the median eminence cytosol is, therefore, of limited capacity.

C) Binding affinity

Specificity of progesterone-binding protein in the median eminence cytosol against other steroid hormones was assayed. At first, the cytosol was incubated with 10^{-9} M labeled progesterone and 10^{-7} M unlabeled steroids. As shown in Table 1, replacement of labeled with unlabeled progesterone was almost complete, whereas no replacement was observed either with corticosterone or with estradiol-17β. After that, the cytosol was incubated with either 10^{-9} M ^{3}H-progesterone or 10^{-9} M ^{3}H-corticosterone, and then passed through a millipore filter. It was observed that 80—90% of the labeled progesterone was bound to the cytosol, whereas only 5—10% of the labeled corticosterone was bound. The binding protein in the median eminence has, therefore, a high affinity for progesterone.

These *in vitro* results are the first to demonstrate the presence of cytoplasmic progesterone-binding complexes, or progesterone receptors in the hypothalamus of female rats. Characterization of the binding component under observation indicates that it is a high-affinity limited-capacity protein which is compatible with a "specific" progesterone receptor. These results also support previously described observations of preferential uptake of the hormone *in vivo* by the hypothalamus of female and male rats. Furthermore, *in vitro* studies have indicated that progesterone is mainly bound to macromolecular protein in the median

eminence cytosol.

Estrogen can cause increased vascular permeability to plasma protein (1). In the present *in vitro* study the rats were treated with estrogen prior to experimentation. There is, therefore, a possibility that progesterone-binding protein from the median eminence cytosol was contaminated with plasma proteins, especially CBG, entering through blood capillaries. In fact, Milgrom and Baulieu (11) reported an increase in progesterone binding in rat unterine cytosol with estrogen treatment. Toft and O'Malley (22) also reported that estrogen treatment altered the progesterone-binding property of chick oviduct cytosol with changes in their sedimentation of 4—5 S to 6—8 S on a sucrose gradient. Furthermore, progesterone-binding proteins which are similar to CBG, but separable from it, have been noted in guinea pig serum (2), in chick oviduct cytosol (21) and guinea pig uterus (12). However, Milgrom and Baulieu (11) have presented persuasive evidence that CBG-like protein in the rat uterine cytosol which appears after estrogen treatment is, in fact, an intracellular component rather than an experimental contaminant from plasma or interstitial fluid. The present *in vitro* study was not concerned with separating progesterone-binding proteins from CBG in the median eminence cytosol, but it clearly demonstrated that progesterone-binding protein did not bind corticosterone, nor was its ability to bind the hormone diminished by corticosterone. These findings strongly deny the possibility in question.

Concerning a physiological role of progesterone in the brain tissue, Dörner and Döcke (3) reported on the alteration of rat ovarian functions after implantation of this hormone into the median eminence. They suggested that this could be a result of the hormone action on production of hypothalamic gonadotrophin releasing hormones in the tissue. Seiki and Hattori (19) and Seiki et al. (20) have observed an increased protein synthesis in the arcuate-ventromedial nuclei in the median eminence of ovariectomized rats injected with progesterone, and referred to the stimulatory effect of this hormone on the production of gonadotrophin releasing hormones in the tissue. With these reports in mind, the present results demonstrating preferential uptake and binding of progesterone in the median eminence may support the concept that the steroid hormone is bound to specific cytoplasmic receptor protein and then the hormone-receptor complexes are transferred to the nucleus where alterations of cellular functions are initiated (4, 7, 8).

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Addentum

In the course of this study an attempt was made in our laboratory to extract a nuclear receptor for progesterone in the ovariectomized rat hypothalamus. Briefly, 30 rats pretreated with estradiol benzoate were sacrificed by decapitation, and the hypothalamic tissues were dissected

out as noted in the text. Each separate tissue was pooled and incubated in Eagle's medium containing 10 μc ^{3}H-progesterone (sp. act. 80 Ci/mM). The incubate was homogenized and centrifuged at $800 \times g$ for 10 min. The $800 \times g$ pellet was filtered through clean nylon gauze and washed with ice-cold 0.25 M sucrose-Tris-HCl (pH 7.4). A nuclear extract was prepared by extraction of the pellet with 0.4 M KCl-Tris-HCl (pH 8.4) for 1 hr at 4°C, followed by centrifugation for 1 hr at 4°C. The extract was then applied on a Sephadex G-200 column.

The Sephadex assay of the extract from the median eminence yielded a protein-bound radioactivity peak which was eluted faster than γ-globulin. The nuclear extract from the remainder of the hypothalamus, on the other hand, did not contain any bound radioactivity peaks.

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