peptide T11 in bovine MBP (*10*) with the addition of Tyr and Gln. It was located just to the left of peptides CNBr 6–8a and 6–6 (Fig. 2). The remaining tryptic peptides in the CNBr 13K peptide were apparently homologous with those in bovine MBP; however, peptides 13–5 and 13–11 were not completely separated.

Therefore, our data show that 21.5K MBP differs from 18.5K MBP by the addition of approximately 30 amino acid residues at the equivalent of residue 57 in bovine 18.5K MBP. As the peptides were hydrolyzed for only 24 hours and as prolonged hydrolysis of 21.5K MBP indicated additional Val residues, it is possible that there could be Val-Val sequences in peptides 6–8c and 21.5–14 because such sequences are resistant to hydrolysis (*5*).

It is likely that the difference in structure between 17K MBP and 14K MBP is due to a similar insert of approximately 30 amino acids as peptide maps of 17 and 21.5K MBP (*1*) showed peptides clearly similar in location to peptides 21.5–14, 6–8a, 6–8b, and 6–8c. Moreover, results on the incorporation of ^{35}S-labeled methionine into mouse 21.5K and 17K MBP's indicated that an additional methionine was present in these proteins (*4*).

Originally, Barbarese *et al.* (*1*) proposed that the additional 30 amino acids were attached at the NH_2-terminal end of 21.5K MBP and that these were removed during metabolic processing to produce 18.5K MBP. The evidence presented for the position of the insert supports the conclusion, from studies on the synthesis of MBP (*3*), that 21.5K MBP cannot be a metabolic precursor of 18.5K MBP.

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8. Analyses were performed on two different Dionex amino acid analyzers and the peak in the hydrolyzate was compared with authentic ornithine. Approximately 1 mole of ornithine was found per mole of 21.5K MBP. No ornithine was found in hydrolyzates of any of the peptides. Free ornithine was released from 21.5K MBP by digestion with trypsin, clostripain, pepsin, and *Staphylococcus aureus* V8 protease. Ornithine has been reported in hydrolyzates of two other proteins [K. Sletten, I. Aakesson, J. O. Alvsakar, *Nature (London) New Biol.* **231**, 118 (1971); A. K. Allen and A. Neuberger, *Biochem. J.* **135**, 307 (1973)].
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Estradiol Is Concentrated in Tyrosine Hydroxylase–Containing Neurons of the Hypothalamus

Abstract. *Localization of [^{3}H]estradiol in tyrosine hydroxylase–containing neurons of rat brain was shown by a combined technique of autoradiography and immunohistochemistry. [^{3}H]Estradiol was concentrated in the nuclei of tyrosine hydroxylase–containing neurons in the nucleus arcuatus, nucleus periventricularis hypothalami, and the zona incerta. These results suggest that estradiol acts directly on dopamine-producing neurons of the tuberoinfundibular system and incertohypothalamic system.*

Tuberoinfundibular dopamine-producing neurons are involved in the regulation of prolactin secretion and gonadotropin secretion (*1*). Dopamine has an inhibitory effect on prolactin release (*2*), but its effect on gonadotropin secretion is not clear (*3*). The association of dopamine terminals with luteinizing hormone–releasing hormone (LHRH) terminals in the palisade zone of the median eminence suggests an interaction between dopamine and LHRH (*4*). The activity of the dopamine neurons changes during the estrous cycle of the rat, being highest during diestrus and lowest during proestrus (*5*). Prolactin stimulates dopamine turnover in the median eminence (*6*). Autoradiographic techniques have revealed that estradiol-concentrating neurons are localized in arcuate and periventricular nuclei of the hypothalamus (*7*), where dopamine neurons and terminals exist (*8*). Applying a combined technique of autoradiography and immunohistochemistry (*9*), Sar and Stumpf showed that [^{3}H]estradiol is concentrated by the central noradrenergic neurons in the lower brainstem of the rat (*10*). I used the same technique to locate both [^{3}H]estradiol and antibodies to the enzyme tyrosine hydroxylase in the same brain section. The results indicate that dopamine neurons of the tuberoinfundibular system as well as of the incertohypothalamic system are target cells for estrogen.

Seven adult female Holtzman Sprague-Dawley rats were ovariectomized and 48 hours later were given intravenous injection of 17β-[2,4,6,7-^{3}H]estradiol (specific activity 105 Ci/mmole) at 0.5 μg per 100 g of body weight. Fifteen minutes before the administration of [^{3}H]estradiol, two of the ovariectomized rats each received unlabeled 17β-estradiol at 100 times the amount of the labeled substance. Rats were decapitated 1 hour after injection of [^{3}H]estradiol. The forebrain and midbrain were dissected, placed on a tissue holder, and frozen in liquefied propane (−180°C). Serial frozen sections (4 μm) were thaw-mounted on slides coated with photographic emulsion (Kodak NTB3) and stored at −15°C for autoradiographic exposure (*11*). After photographic exposure, the slides were fixed in 4 percent paraformaldehyde solution for 30 seconds at 4°C; rinsed briefly with phosphate-buffered saline (PBS), *p*H 7.5; and developed (Kodak D-19 developer, fixation with Kodak fixer). After being rinsed with PBS, the autoradiographic slides were processed for immunoperoxidase stain-

ing with antibodies to bovine tyrosine hydroxylase produced in rabbit (*12*). Autoradiograms were incubated with rabbit antiserum to bovine tyrosine hydroxylase (1:500 or 1:1000, diluted in PBS) for 24 to 48 hours at 4°C. The specificity of immunoperoxidase staining was established by incubating the brain autoradiograms with normal rabbit serum or with antiserum to dopamine β-hydroxylase. The immunostaining of thaw-mounted autoradiograms has been described (*9*).

Autoradiograms of brain that had been stained immunohistochemically with antiserum to tyrosine hydroxylase showed tyrosine hydroxylase–containing cells in the periventricular nucleus, arcuate nucleus, and certain regions of subthalamus, as well as in the region of the substantia nigra and the ventral tegmental area. The distribution of neurons containing tyrosine hydroxylase is comparable to the distribution of the dopamine neuronal system observed by histofluorescence and by immunohistochemistry (*8, 13*). The tyrosine hydroxylase–containing neurons that are located in the arcuate and periventricular nucleus belong to the A12 group (*13*). These cells are found in the rostral half of the arcuate nucleus, with some scattered cells in the caudal half of the nucleus and a portion of the periventricular nucleus situated dorsal to the arcuate nucleus. The periventricular group extends from the preoptic region to the median eminence. When autoradiograms were stained with antiserum to dopamine β-hydroxylase or normal rabbit serum, no cells were stained in these regions.

Tyrosine hydroxylase–containing cells in the arcuate nucleus and in the hypothalamic periventricular nucleus show radioactivity concentrated in their nuclei after [^{3}H]estradiol administration (Fig. 1, a and b, and Fig. 2). The percentage of tyrosine hydroxylase–containing cells labeled with [^{3}H]estradiol varies considerably in different regions. About 10 to 15 percent of the labeled cells in the arcuate nucleus contain tyrosine hydroxylase, whereas in the periventricular nucleus, 30 to 40 percent of the tyrosine hydroxylase–containing cells are labeled. The tyrosine hydroxylase–containing cells that are located in the dorsal part of the third ventricle do not show a concentration of radioactivity. Many cells in arcuate and periventricular nuclei concentrate [^{3}H]estradiol in their nuclei but do not stain immunohistochemically with antiserum to tyrosine hydroxylase. Tyrosine hydroxylase–containing cells in the dorsal hypothalamus, especially in the zona incerta, that belong to A13 group are weakly labeled in comparison with the cells of the A12 cell group (Figs. 1c and 2). In the A13 group, 15 to 20 percent of the cells are labeled. Scattered cells in thalamic and subthalamic areas, as well as the cells of the substantia nigra (group A9) and ventral tegmental area (group A10), do not show a concentration of radioactivity in their nuclei (Fig. 1d). Cells belonging to group A14 are not labeled, but a few tyrosine hydroxylase–containing cells of group A11 are labeled. The localization of [^{3}H]estradiol is specific, since administration of an excess of unlabeled estradiol abolishes the nuclear uptake.

Combined autoradiographic and immunohistochemical studies provide direct evidence that certain tyrosine hydroxylase–containing neurons in rat hypothalamus are targets for estradiol. These include the dopamine neurons of tuberoinfundibular system as well as the group A13 neurons of the incertohypothalamic system. The results agree well with the earlier observation that estradiol exerts a selective and specific effect on the tuberoinfundibular dopamine–producing system (*14*). In the female rat, for example, the activity of the tuberoinfundibular dopamine neurons is decreased after ovariectomy and increased again when the animal is treated with estradiol or testosterone. Even in high doses, glucocorticoids have no significant effect on the activity of the tuberoinfundibular dopamine neurons (*14*). In castrated males, estradiol and testosterone treatment produces a marked increase in amine depletion in the dopamine nerve terminals of the median eminence but not in the nigrostriatal dopamine nerve terminals (*14*). Administration of 17β-estradiol after ovariectomy produces an increase in tyrosine hydroxylase activity in the median eminence and a decrease in monoamine oxidase activity in the basomedial hypothalamus (*15*).

Estrogen treatment in rats produces a marked increase in the concentration of serum prolactin as well as an increase in the concentration of pituitary stalk plasma dopamine (*16*). The estrogen-induced increase of dopamine concentration may be the result of an increased secretion of prolactin and a subsequent feedback effect of prolactin on the release of dopamine from tuberoinfundibular dopamine neurons (*17*). The increased dopamine turnover after estradiol administration is mediated in part by an increase in prolactin secretion (*14, 16, 17*). Changes in tyrosine hydroxylase activity and catecholamine turnover within the median eminence may, however, occur without

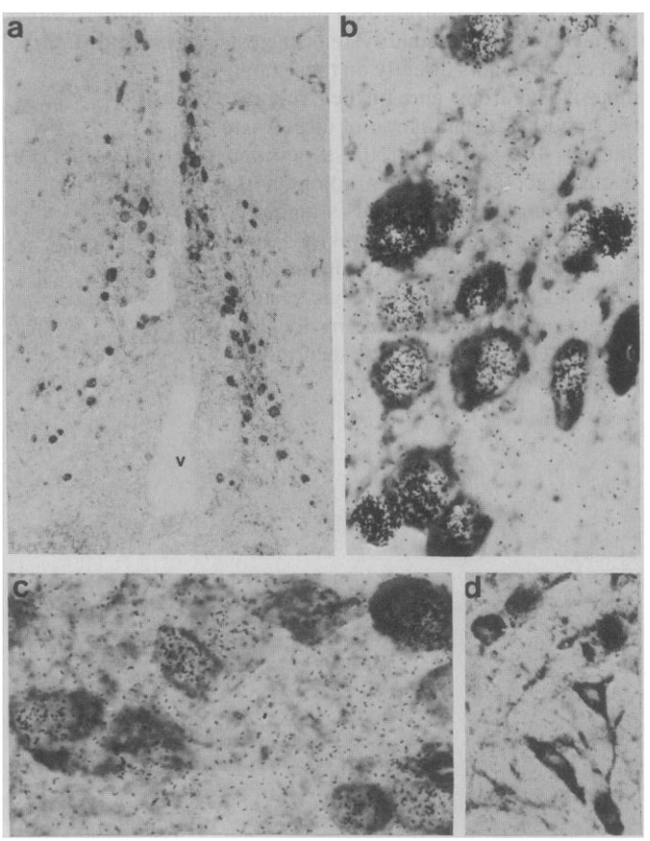

Fig. 1. Thaw-mounted autoradiograms of rat brain 1 hour after injection of [^{3}H]estradiol stained with antiserum to tyrosine hydroxylase (1:500) by the immunoperoxidase method. Radioactivity is concentrated in nuclei of tyrosine hydroxylase–containing neurons in arcuate and periventricular nuclei (a and b) and in group A13 cells of the zona incerta (c), but not in the dopamine neurons of group A9 cells of the substantia nigra (d). *V*, third ventricle. Exposure time, 360 days. Sections, 4 μm thick. Magnifications: ×140 (a), ×890 (b and c), and ×350 (d).

producing changes in prolactin secretion (*18*). In hypophysectomized and castrated rats, estrogen increases dopamine synthesis and turnover in the median eminence (medial and lateral palisade zone), suggesting a direct action of estradiol on the brain (*19*). Estrogen at physiological concentration also produces an increase in dopamine turnover in the medial and lateral palisade zone of the median eminence. In this situation, it is unlikely that hypersecretion of prolactin is responsible, since no significant increase of prolactin secretion can be observed in animals treated with estrogen alone or in combination with progesterone (*19*).

A large number of neurons in the nucleus periventricularis hypothalami and nucleus arcuatus are labeled with [^{3}H]estradiol, but they do not stain with antibodies to tyrosine hydroxylase. These estrogen-sensitive neurons have not yet been characterized. Only a few of these radioactively labeled neurons are identified as somatostatin-producing cells (*20*). Other peptide-producing neurons located in these regions include opiate peptides (enkephalins, dynorphin, and β-endorphin), vasoactive intestinal peptide, and cholecystokinin. Future research is therefore needed to determine whether some of these peptide-producing neurons are targets for estradiol.

The finding that estrogen is localized in tuberoinfundibular dopamine–producing neurons and in group A13 neurons of the incertohypothalamic system suggests that estradiol acts directly on these dopaminergic neurons and further supports the hypothesis that estradiol directly influences the activity of these neurons. Such direct action of estradiol on the central noradrenergic and γ-aminobutyric acid–producing neurons has been reported (*10, 21*). Estrogen-induced effects on the other dopaminergic systems may be indirect (*22*), since no localization of [^{3}H]estradiol can be seen in neurons of the mesolimbic and nigrostriatal systems. Whether estrogen has a stimulatory or an inhibitory effect on dopamine neurons is not established, although an inhibitory feedback effect of estrogen on gonadotropin secretion mediated through tuberoinfundibular dopaminergic neurons has been suggested (*1*). The mechanism of estrogen's action on tuberoinfundibular dopaminergic neurons has not been determined.

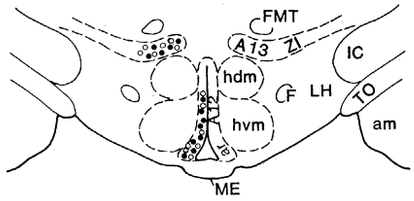

Fig. 2. Schematic drawing showing the distribution of tyrosine hydroxylase–containing neurons (●) that concentrate [^{3}H]estradiol and (○) those that do not concentrate [^{3}H]estradiol in rat hypothalamus (frontal plane). Abbreviations: *am*, nucleus amygdaloideus medialis; *ar*, nucleus arcuatus, *A12*, dopamine neurons in arcuate and periventricular nuclei; *A13*, dopamine neurons of incertohypothalamic system; *F*, columna fornicis; *FMT*, fasciculus mamillo thalamicus; *hdm*, nucleus dorsomedialis hypothalami; *hvm*, nucleus ventromedialis hypothalami; *IC*, internal capsule; *LH*, lateral hypothalamic area; *ME*, median eminence; *TO*, tractus opticus; and *ZI*, zona incerta.

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