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Estradiol and Testosterone in Specific Regions of the Human Female Brain in Different Endocrine States

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Post-mortem concentrations of estradiol and testosterone were measured in 17 brain areas, serum and fat in 6 fertile and 5 postmenopausal women. Steroid concentrations were measured with radioimmunoassay after extraction of brain tissue with ethanol and purification with celite chromatography. There were regional differences in brain concentrations of both steroids. The highest levels of estradiol and testosterone were noted in the hypothalamus, preoptic area and substantia nigra. These findings may assist in the interpretation of functional animal studies where the hypothalamus-preoptic area and the nigrostriatal dopamine system have proved to be target areas for estradiol. When compared to postmenopausal women, estradiol concentrations were significantly higher in the brains of fertile women, which indicates that peripheral serum levels of estradiol are reflected in the brain. This study has yielded information about steroid levels in different endocrine states and could provide a frame of reference for studies of estradiol and testosterone mediated effects on the central nervous system.

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INTRODUCTION

It has been satisfactorily established that sex steroid hormones have direct effects within the brain. Estradiol and progesterone affect hypothalamic secretion of releasing hormones in the female monkey [1, 2] and testosterone appears to regulate sexual behavior in this species [3]. In addition, estradiol increases brain excitability, while progesterone and some of its metabolites have the reverse effect, acting as anticonvulsants [4, 5]. Elevated levels of estradiol are associated with activating effects on mood and alertness [6, 7], but also on sensimotor function [8] and locomotor activity [9]. In addition, attentional mechanisms and short term memory appear to be enhanced following cyclic elevation in estradiol [10, 11]. There are several possible mechanisms that could mediate the excitatory effect of estradiol in the brain. Estradiol is known to potentiate glutamate-induced excitation in the cerebellum [12] but also to decrease γ-aminobutyric$[GABA]_A$-receptor density in several brain regions [13].

The uptake of steroids in the brain has previously been studied in female rats, in which high estradiol and testosterone uptake was noted in the hypothalamus, hippocampus and amygdala [14, 15]. Specific receptors for estradiol and testosterone have been detected in the rat brain, located intracellularly as well as binding to synaptic plasma membrane [16–18]. In rhesus monkeys, an accumulation of estradiol has been noted in specific sites of the hypothalamus and amygdala [19, 20]. Local synthesis of estradiol from testosterone was seen in the hypothalamus and limbic system [21] and this aromatization seems to be closely linked to testosterone receptor activity [22]. In two previous studies of sex steroids in specific regions of the human brain the subjects were men and prepubertal or postmenopausal women and only a few brain regions were investigated [23, 24]. Low concentrations of progesterone, estradiol, testosterone and some other androgens were found. In the present study, 6 fertile and 5 postmenopausal women were investigated.

Earlier studies from this laboratory have shown that steroid concentrations in the rat brain are affected by autolysis, as previously observed regional differences in progesterone concentration became less prominent

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Table 1. *Clinical and pathological data*

Case number	Age (y)	Cause of death	Postmortal time (h)	Cycle phase*
1.	38	Perforating ulcer	24	Luteal
2.	21	Car accident	69	Luteal
3.	18	Status asthmaticus	42	Luteal
4.	25	Suicide by hanging	25	Luteal
5.	42	Suicide by hanging	48	Amenorrhoic
6.	75	Myocardial infarction	24	Postmenopausal
7.	79	Myocardial infarction	69	Postmenopausal
8.	59	Respiratory insufficiency	40	Postmenopausal
9.	85	Congestive heart failure	27	Postmenopausal
10.	65	Carcinoma of the gallbladder	50	Postmenopausal
11.	19	Train accident	67	—**

*Based on macroscopic and histological investigations of endometria and ovaries.

**Treated with triphasic oral contraceptives with 30–40 μg of ethinylestradiol and 50–125 μg of levonorgestrel.

[25]. Both time between death and dissection and the storage temperature were of importance. It is conceivable that steroid levels in the human brain are affected in the same way, although the time frame is different because of difference in body mass between man and rat. Thus, in the present study, we have matched fertile and postmenopausal subjects with regard to the time between death and autopsy, assuming that the brain cooling curves were about the same, since the storage temperatures were the same for all cadavers.

The purpose of the present study was to describe the regional distribution of estradiol and testosterone in the adult female brain and the hypothesis that endocrine status is reflected in the brain was tested.

MATERIALS AND METHODS

Human tissues

Brains were removed at autopsy and abdominal fat tissue and blood samples were taken from 6 fertile and 5 postmenopausal female cadavers. 10 of them were matched with regard to postmortal time and storage temperature. None of them had any history of neurological disease or recent treatment with steroids. The ovaries and endometria were examined histologically to confirm endocrine status. Age, cause of death, cycle phase and time between death and autopsy are summarized in Table 1.

Tissue samples from 17 different brain areas (shown in Figs 1 and 2) were dissected out immediately according to the following procedures.

Frontal cortex. Grey matter from the most frontal part of the frontal lobe (Brodmann area 19) was taken.

Temporal cortex. Grey matter from Brodmann areas 21, 22 and 38 was taken.

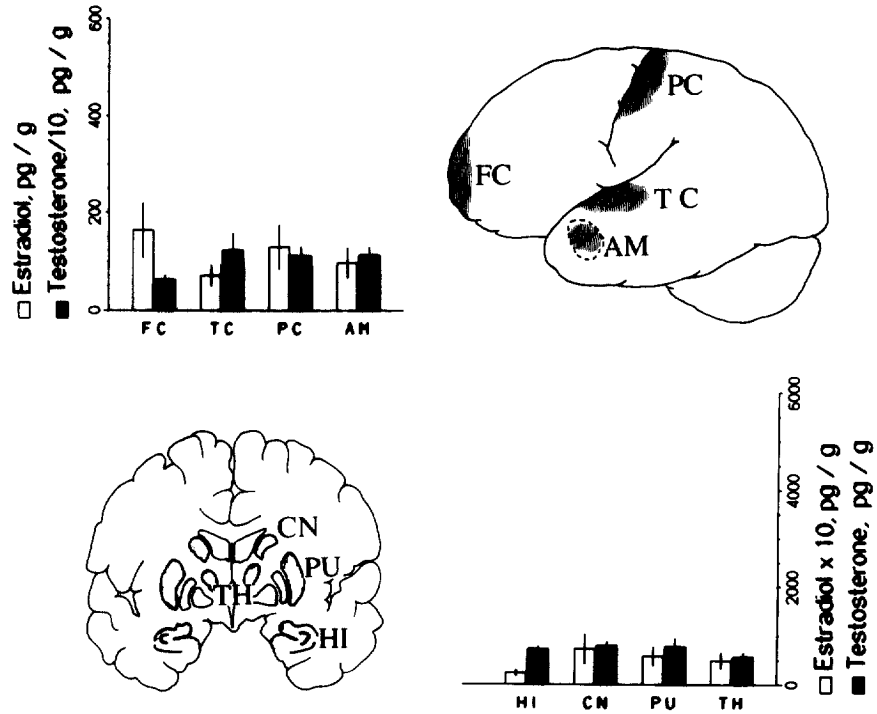

Fig. 1. Concentrations (mean (SEM), pg/g) of estradiol and testosterone in specific regions of the human female brain ($n = 10$). Note that in top diagram testosterone concentrations are divided by 10 and in bottom diagram estradiol concentrations are multiplied by 10. FC, frontal cortex; TC, temporal cortex; PC, parietal cortex; AM, amygdala; HI, hippocampus; CN, caudate nucleus; PU, putamen; TH, thalamus.

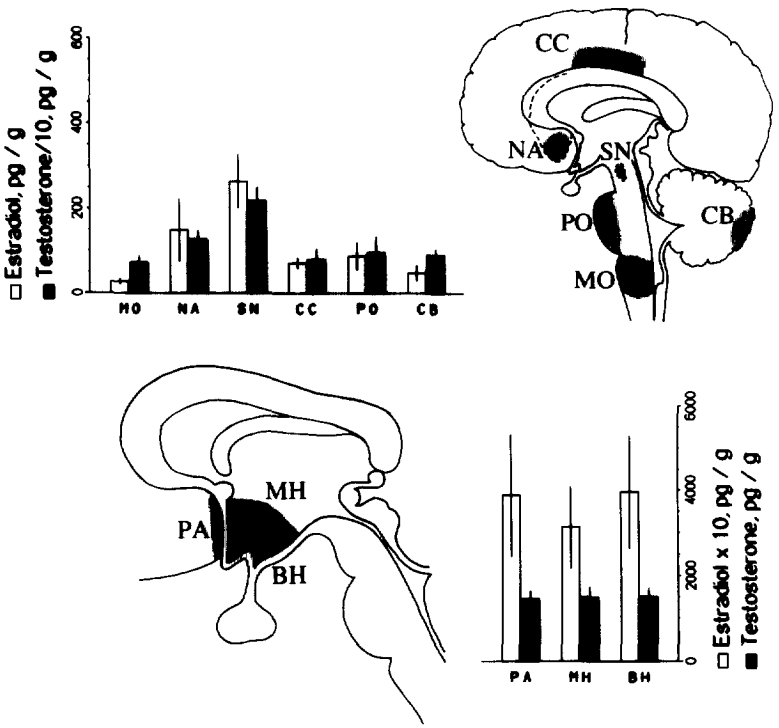

Fig. 2. Concentrations (mean (SEM), pg/g) of estradiol and testosterone in specific regions of the human female brain ($n = 10$). Note that in top diagram testosterone concentrations are divided by 10 and in bottom diagram estradiol concentrations are multiplied by 10. MO, medulla oblongata; NA, nucleus accumbens; SN, substantia nigra; CC, cingulate cortex; PO, pons; CB, cerebellum; PA, preoptic area; MH, medial hypothalamus; BH, basal hypothalamus.

Parietal cortex. Grey matter from the postcentral gyrus (Brodmann areas 1, 2, 5 and 7) was taken.

Cingulate cortex. Grey matter from the middle part of the cingulate gyrus (Brodmann areas 23, 24 and 31) was taken.

Hypothalamus and preoptic area. After removing the pituitary, the optic chiasm and terminal lamina were divided sagitally and an additional sagittal incision between the mamillary bodies exposed the third ventricle. The hypothalamus was obtained with incisions posteriorly to the mammillary bodies, superiorly along the hypothalamic sulcus, anteriorly behind the anterior commissure and terminal lamina and laterally about 5 mm from the wall of the third ventricle. The hypothalamus was then divided into a basal part including the infundibular, supraoptic, ventromedial and mamillary nuclei and a medial part including the paraventricular, dorsomedial and posterior nuclei. The preoptic area was obtained with another transverse incision just anteriorly to the anterior commissure and optic chiasm.

Pons. A horizontal incision, about 5 mm thick, was made through the pons between the cerebellar peduncles and the medulla oblongata.

Medulla oblongata. Obtained with an incision just below the pons and a caudal incision below the olives.

Cerebellum. Grey matter was taken from the most dorsal part of the superior and inferior semilunar lobes.

Substantia nigra. Identified with adjacent horizontal incisions through the cerebral peduncles, and the dark tissue (pars compacta) was excised.

Thalamus. Most of the thalamic nuclei were included by a circular incision from the surface of the third ventricle, medially to the cauda of the caudate nucleus and inferiorly along the hypothalamic sulcus. A horizontal incision was made about 5 mm below the top of the thalamus.

Nucleus accumbens. Defined as the ventro-medial part of the head of the caudate nucleus and down to the white matter in the internal capsule.

Caudate nucleus. The nucleus was excised from the floor of the lateral ventricle in its full length.

Hippocampus. Excised in its full length from the floor of the temporal horn of the lateral ventricle with a horizontal incision along the hippocampal sulcus.

Amygdala. A transverse incision through the temporal lobe in front of the head of the hippocampus was made to expose the amygdala. A central part of the complex of nuclei was taken.

Putamen. Defined as the lateral part of the basal ganglia, located between the external capsule and globus pallidus. Most of the grey matter was excised.

Table 2. Concentrations of estradiol (mean (SEM), pg/g) in human female brain tissue, abdominal fat and serum (n = 10). Multiple range analysis using ANOVA followed ad hoc by LSDT at 95% level. Regions differ significantly when not marked as homogeneous groups with an × in the same vertical column

Region	Mean (SEM), pg/g	Homogeneous groups			
Hippocampus	23.3 (7.3)	×			
Medulla oblongata	27.6 (6.8)	×			
Cerebellum	47.9 (19.0)	×			
Thalamus	49.2 (17.0)	×			
Putamen	58.1 (20.0)	×			
Cingulate cortex	71.4 (14.0)	×			
Temporal cortex	71.5 (22.0)	×			
Caudate nucleus	72.9 (32.0)	×			
Pons	87.6 (34.0)	×	×		
Amygdala	97.2 (32.0)	×	×		
Parietal cortex	129 (45.0)	×	×		
N. Accumbens	149 (75.0)	×	×	×	
Frontal cortex	163 (56.0)	×	×	×	
Substantia nigra	265 (64.0)		×	×	×
Medial hypothalamus	312 (97.0)			×	×
Preoptic area	388 (144)				×
Basal hypothalamus	396 (133)				×
Fat tissue	169 (40.0)				
Serum	302 (110)				

Tissues and blood serum were stored at −70°C until analyzed.

Extraction procedure

The tissue samples were extracted with ethanol (AB Svensk Sprit, Sundsvall, Sweden) for 7 days. The recovery of steroid by this method is 100% [26]. The blood serum was extracted with diethyl ether (recovery 90%).

Celite chromatography

The two steroids were separated with celite column chromatography [27]. Glass columns (inner diameter 5 mm) were tightly packed with celite (Mansville, Denver, U.S.A.), preheated at +600°C for 12 h and saturated with ethylene glycol, to a height of 50 mm. Nitrogen was used to percolate all solvents through the columns. For recovery measurements purified ^{3}H-steroids were added to the extracts before evaporation. The samples (40–70% of the extracts) were dissolved in 1 ml of isooctane saturated with ethylene glycol before chromatography. Isooctane (4 ml) and 3.5 ml of isooctane:ethylacetate (95:5) were percolated through the columns and subsequently testosterone was eluted with 3.5 ml of isooctane:ethylacetate (85:15) and estradiol with another 3.5 ml of isooctane:ethylacetate (50:50). The samples were evaporated under nitrogen and dissolved in ethanol.

Hormone assay

The concentrations of estradiol and testosterone in tissue and serum extracts were measured with radioimmunoassay, described in detail earlier [28]. The estradiol antiserum was raised against a 17β-estradiol–BSA-antigen and obtained from Miles Yeda Ltd, Rehavof, Israel. The testosterone antiserum was raised against a testosterone-3-oxime-BSA-antigen and obtained from the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden. The coefficient of variation within and between assays were 8 and 10% for estradiol and 5 and 5% for testosterone. The cross-reaction in the testosterone assay was less than 0.4% for a number of androsten and androstan steroids. 5α-dihydrotestosterone had a cross-reactivity of 40% and androstenedione 1.8%. However, those two steroids are separated from testosterone in the celite chromatography [29].

Statistical methods

For comparison between brain areas one-way ANOVA followed *ad hoc* by a least significant difference test (LSDT) was used. For comparison between groups two-way ANOVA was used. A multiple regression analysis was used between serum and brain regions. Statgraph Statistical Package was used for analysis.

RESULTS

Statistically significant differences between brain regions in all subjects were found for both estradiol and testosterone ($F(16,155) = 3.81$, $P < 0.001$ and $F(16,163) = 6.02$, $P < 0.001$ respectively). Steroid concentrations in different brain regions are shown in

Table 3. Concentrations of testosterone (mean (SEM), pg/g) in human female brain tissue, abdominal fat and serum (n = 10). Multiple range analysis using ANOVA followed ad hoc by LSDT at 95% level. Regions differ significantly when not marked as homogeneous groups with an × in the same vertical column

Region	Mean (SEM), pg/g	Homogeneous groups				
Thalamus	568 (84.0)	×				
Frontal cortex	633 (98.0)	×				
Medulla oblongata	726 (163)	×				
Hippocampus	726 (62.0)	×				
Putamen	783 (174)	×	×			
Caudate nucleus	800 (88.0)	×	×			
Cingulate cortex	801 (250)	×	×			
Cerebellum	907 (142)	×	×	×		
Pons	962 (390)	×	×	×		
Parietal cortex	1120 (193)		×	×	×	
Amygdala	1140 (165)		×	×	×	
Temporal cortex	1240 (347)		×	×	×	
N. Accumbens	1270 (219)			×	×	
Preoptic area	1440 (195)				×	
Medial hypothalamus	1490 (262)				×	
Basal hypothalamus	1500 (213)				×	
Substantia nigra	2200 (326)					×
Fat tissue	1470 (575)					
Serum	1040 (294)					

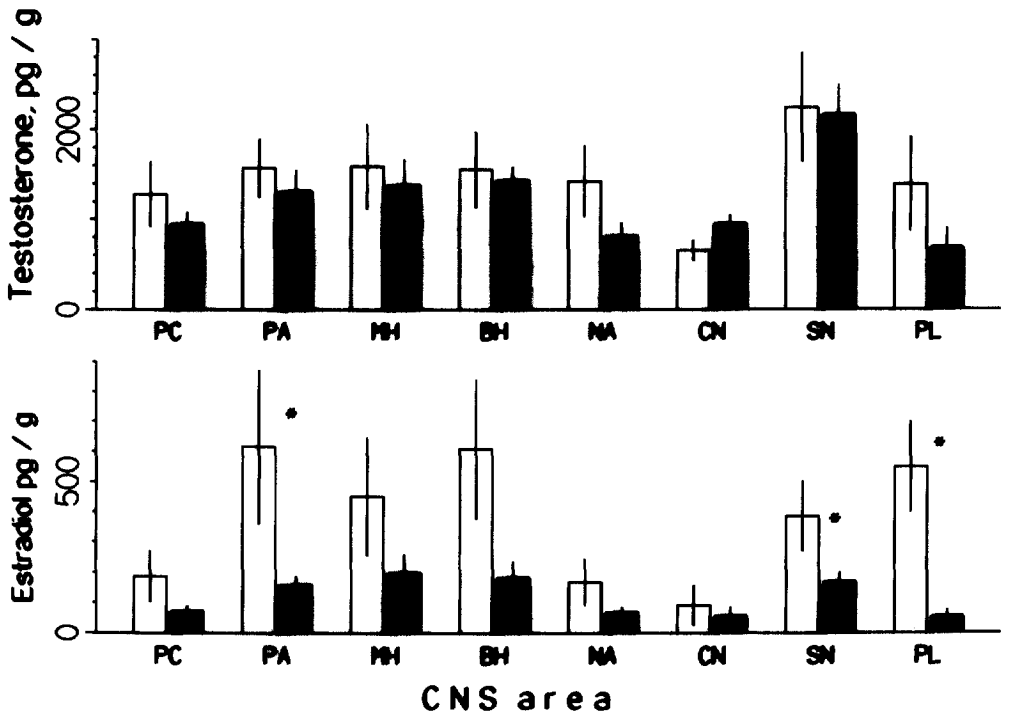

Fig. 3. Comparisons of testosterone and estradiol concentrations (mean (SEM), pg/g) in some specific brain regions of women with high ($n = 5$) and low ($n = 5$) serum steroid levels. Differences tested by the Mann–Whitney U-test, *$P < 0.05$. PC, parietal cortex; PA, preoptic area; MH, medial hypothalamus; BH, basal hypothalamus; NA, nucleus accumbens; CN, caudate nucleus; SN, substantia nigra; and PL, plasma.

Figs 1 and 2. Statistically significant differences are presented in Table 2 and 3. The highest concentrations of estradiol were found in the hypothalamus and preoptic area and the highest testosterone levels were noted in the substantia nigra, hypothalamus and preoptic area. Differences between fertile and postmenopausal women were statistically significant for estradiol but not for testosterone ($F(1,155) = 7.87$, $P < 0.01$ and $F(1,163) = 3.86$, N.S., respectively). A comparison between women with high and low serum steroid levels in some specific brain regions is presented in Fig. 3. Significant differences were seen in the preoptic area and substantia nigra for estradiol, whereas no significant differences were noted for testosterone. The reason serum steroid concentrations were used as a basis for defining categories instead of ovarian endocrine state was that one of the postmenopausal women had very high serum estradiol levels, probably due to congestive heart disease [30]. In addition, one of the fertile women had unusually low serum concentrations due to amenorrhea. One additional subject (a young woman using oral contraceptives) was assayed for steroids. Her serum estradiol concentration was low (60 pg/ml), as were the brain concentrations which ranged from 1.5 to 162 pg/g. No correlation between serum and brain concentrations for estradiol or testosterone were found. The ratio of estradiol to testosterone differed significantly between brain areas ($F(16,138) = 1.84$, $P < 0.05$) and was highest in the hypothalamus, frontal cortex and preoptic area.

DISCUSSION

The results of this study are partly in agreement with previous animal studies on the regional brain distribution of steroid hormones. The two steroids were found in comparably high concentrations in the hypothalamus, corresponding to results when studying rats and monkeys [14, 15, 19]. In the few (2 + 2) adult women reported on previously, only 3 and 4 brain areas were studied. The highest levels of estradiol and testosterone were noted in the amygdala, whereas the concentrations in the hypothalamus were similar to those in the cerebral cortex. However, in most of the areas studied, estradiol and testosterone were not detectable [23, 24]. This is not in agreement with our results. One possible reason for this might be the sensitivity of the methods. Several samples in these previous studies showed non-detectable concentrations in regions where we found measurable concentrations of both steroids. However, in other brain regions, steroid levels obtained in the present study were equal to or less than levels measured in corresponding regions in these previous studies. Also, there were fewer cases in these studies and the subjects were males or postmenopausal females.

In the present study, the highest concentrations of both estradiol and testosterone were seen in the same brain regions, i.e. the hypothalamus, preoptic area and substantia nigra. In addition, the ratio of estradiol to testosterone was high in the hypothalamus and preoptic area. We find that interesting since high aromatase activity has been found in the hypothalamus and preoptic area of rats [31]. The relatively high levels of estradiol in the substantia nigra are also of interest since estradiol is known to increase dopamine uptake in the nigrostriatal dopamine system in rats [32]. Generally speaking, brain concentrations of estradiol differed with different serum estradiol concentrations. This finding indicates that brain concentrations are a result of peripheral production as well as synthesis within the brain. This hypothesis is further strengthened by the fact that brain testosterone levels did not vary with serum testosterone levels. The levels of testosterone did not differ significantly between the two subgroups. The lack of difference is probably not due to cross-reactivity with other androgens, since the antibody used had a high specificity for testosterone [29]. The only steroid with a significant cross-reactivity is 5α-dihydrotestosterone which is separated from the sample in the celite chromatography. Brain concentrations of testosterone seem to be rather stable and in the present study we have only investigated women with no difference in serum testosterone concentrations. In fact, in an earlier study of a few cases the brain concentrations did not differ much even between men and women [24]. The comparatively low estradiol concentrations in serum and brain in the subject who had used oral contraceptives might reflect the inhibitory actions of synthetic steroids on the endogenous secretion of gonadal steroids.

Studies of steroid hormone concentrations in the postmortal human brain are associated with great difficulties since conditions obviously cannot be subject to the same degree of control as in animal experiments. Concerning the subjects of the present study, one might expect the highest steroid concentrations to be up to 50% lower than *in vivo* and the lowest concentrations up to 50% higher, since the regional differences in brain concentrations tend to diminish during autolysis [25]. However, we believe that studies like this might provide some useful information. For instance, the physiological range in brain tissue concentrations is of interest when assessing *in vitro* concentrations obtained during specific physiological conditions or pharmacological treatments. In addition, the mapping of gonadal steroid concentrations in the female human brain could provide a frame of reference for comparative studies of pathological conditions such as catamenial epilepsy and the premenstrual tension syndrome [5, 7, 33]. Comparisons between fertile and postmenopausal women could also be of interest regarding the many activating actions of estradiol in the brain (see Introduction). Our findings might contribute to the discussion concerning postmenopausal hormone replacement therapy.

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