

Concentration of Serotonin And 5 Hydroxy Indole Acetic Acid in Discrete Brain Regions of Ovariectomized And Estradiol Treated Female Rats

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Abstract

Back Ground: Estrogen is the sex hormone that causes the development and maintenance female secondary sexual characters. Post menopausal women increasing in vulnerable to depression due to the reduced estrogen production. The activity of estrogen on serotonin has anti depressant characteristics. A higher level serotonin activity is associated with Positive mood while decreased activity is associated with depressive mood. Estrogen boosts the activity of serotonin.

Objectives: To determine the brain serotonin and its major metabolite 5HIAA in ovariectomized rats at different day intervals and after estradiol treatment.

Methodology: Female albino rats of wister strain where ovariectomized and treated with ethanyl estradiol, Brain serotonin and 5HIAA was estimated by Fluoremetric method. Data was analysed by student's t test.

Result: Reduction in serotonin and 5HIAA level in Hypothalamus, striatum and pons medulla after ovariectomy and increased in estradiol treated rats.

Conclusion: Estrogen may be used as effective anti depressant after further studies.

I. Introduction

Estrogen is the sex hormone, that causes the development and maintenance of female secondary sexual characters. Post menopausal women increasingly vulnerable to depression due to the reduced estrogen production. The activity of estrogen on neurotransmitter has anti depressant characteristic¹.(Weissman, 1996). . A higher level of serotonin activity is associated with positive mood while decreased activity is associated with depressive mood. Estrogen boost the activity of serotonin.Estrogen deficiency leads to vasomotor instability (hot flushes and night sweat) while deprivation leads to urogenital atrophy, osteoporosis and tooth loss, atherosclerosis and coronary heart disease and potentially increase the risk of dementias².During menopause women experience hormonal and chemical changes. There is a decrease in the level of estradiol, the principal estrogen of women⁴. Prolonged estrogen deficiency may be associated with impairment in estradiol receptor in the brain. Estradiol receptor may be important in the facilitatory effect that estrogen has on the activity of serotonin and , when the receptors are impaired the estrogen does not work as well and is unable to facilitate the serotonin⁵.

II. Aim Of The Present Study

1. To find out the concentration of serotonin and its major metabolite 5HIAA in different brain regions of rat at different day intervals after ovariectomy.
2. To find the concentration of serotonin and its major metabolite 5HIAA in different brain regions at 24 hours and 36 hours after estradiol treatment to ovariectomized rat.

III. Materials And Methods

Healthy matured female albino rats of wister strain (180-220g) were used in the present investigation. The animals were maintained in a well ventilated and fed with standard balanced rat pellet diet (Gold Mohur Hindis tain liver Ltd. Bombay India) and drinking water was made available ad libitum.

Experimental animals were divided into 6 groups all are female.

Group I Control rats. This group consist of 6 Normal rats.

Group II Ovariectomized rats. Sacrificed after 5 days (n = 6).

Group III Ovariectomized rats. Sacrificed after 10 days (n = 6).

Group IV Ovariectomized rats. Sacrificed after 15 days (n = 6).

Group V Ovariectomized rats treated with 20 µg Ethinyl Estradiol sacrificed after 24 hours (n = 6).

Group VI Ovariectomized rats treated with 20 µg Ethinyl Estradiol sacrificed after 36 hours (n = 6).

3.1. Dissection And Regionation Of Rat Brain

Rats were sacrificed by rapid decapitation, without initial disturbance for substantial changes in brain amines may occur within few minutes, if the animals were disturbed unduly before sacrifice⁸ (Welch and Welch 1968) Anaesthesia was not used because it also alters the brain amines⁷ Vogt 1954, Schanberg et al 1967. The skin from the top of the head was removed with a couple of incisions and cut off the occipital muscles from the occipital bone, revealing the foramen magnum. One blade of the scissors was cautiously introduced into the foramen. The blade was kept parallel to the inner wall of the calvarium and close to it as possible to avoid damaging the brain cuts were made through the bone. The cuts extended rostrally as far as the bregma suture. After two parallel cuts through the bone were made, the bone was lifted forward and its point of attachment rostrally was broken. The head was held more upward and closed scissors were slipped under the frontal lobe and the brain was gently separated from the skull. Optic nerve which leaves the brain base was severed. Finally the brain was taken out of the skull after trigeminal nerves were cut. The brain removed was transferred to an ice cold glass plate. The brain regions were dissected following the procedure of⁹ Golowinski and Iversion (1966). The following regions were separated : cerebellum, Pons-medulla, Hypothalamus, Striatum, Midbrain, and cerebral cortex. All regions were blotted immediately and weighted separately in an electrical monopan balance.

3.2. Fluoremetric Estimation Of Tissue Serotonin And Shiaa¹⁰ By The Method Of Curzon And Green (1970).

All the chemicals used were of Analytical reagent grade and all glass double distilled water was used throughout for preparing various solutions. The excised brains were dissected out into 6 major parts and weighed. The cortex was homogenized in 10 volumes of cold acidified n butanol and all other areas (weighing less than 300mg) were homogenized in 3ml of acidified butanol. The homogenate were centrifuged at 3000rpm for 5 minutes. The clear supernatant was divided into two equal aliquots.

1. To one of the sample in a 15ml centrifuge tube was added 5ml of heptan and 0.4ml of 0.1N HCl containing 0.1% L cystine.

2. The second aliquot was processed similar to the above, step instead of 0.1N HCl containing 0.1% Cystine, 0.4ml of known amount of 5HT internal standard was added.

3. Reagent blank contained 2.5ml of acid butanol 5ml of heptan and 0.4ml of 0.1%N HCl containing 0.1% L cysteine was added.

The all tubes were then shaken for 5 minutes and centrifuged at 3000rpm for 5 minutes.

5ml of the organic phase retained for 5HIAA determination.

To determine 5 HT, 0.1ml samples of the aqueous phase were pipette out into test tubes and added 0.6ml of 0.004 % OPT in 10N HCl. After mixing the tubes were kept in boiling water bath for 15 minutes. The tubes were cooled in water and fluorescence unit were measured for 5HT is 360/470nm. To determine 5HIAA 5ml of organic phase was pipette out into 15ml centrifugal tube. To the sample tube 6ML of 0.5M phosphate buffer was added. In the reagent blank tube, also 6ML of 0.5M phosphate buffer was added. In the standard tube 0.6ml of 5HIAA internal standard was added and processed similar. The above all tubes were shaken for 10 minutes and centrifugal at 300rpm for 3 minutes. 0.2ml of aqueous phase was extracted and pipette out into test tubes and added 0.02ml of 1% cysteine solution, 0.4ml of conc HCl, 0.02ml of 0.1% OPT and 0.02ml of 0.02% periodate solutions were added. The tubes were placed in boiling water for 10 minutes and cooled in water and read at activation 360nm fluorescence 470nm. The concentration of 5HT and HIAA present in the samples were calculated according to the procedure of Ansell and Beeson (1968)³⁰.

$$\frac{A - \text{blank}}{B - A} \times \text{Conc of Std} \times \frac{x}{2.5} \times \frac{x}{y} \times 1 = \mu\text{g/gm of tissue}$$

Where

- A** = fluorescence unit obtained from tissue alone
B = fluorescence obtained from tissue + mixed std
X = Volume of Butanol
Y = Weight of tissue in grams
2.5 = Volume of homogenate used

Statistical Analysis

The values are expressed as mean, S.D. statistically significant variations between the groups arrived at using students 't test'

IV. Results

Table – 1 Effect Of Ovariectomy On 5ht (µg/Gm Of Tissue) Levels In Different Brain Regions Of Female Rats

Brain Regions	Control (Normal Rats)	5 th day after Ovariectomy	10 th day after Ovariectomy	15 th day after Ovariectomy
Hypothalamus	1.342 ± 0.236	1.022 ± 0.20	0.946 ± 0.098	0.8076 ± 0.0219
Striatum	1.086 ± 0.119	1.019 ± 0.138	0.895 ± 0.226	0.6494 ± 0.0109
Mid Brain	0.340 ± 0.126	0.245 ± 0.059	0.288 ± 0.046	0.094 ± 0.009
Pons Medulla	1.461 ± 0.016	0.018 ± 0.098	0.865 ± 0.050*	0.865 ± 0.007**
Cerebral- Cortex	0.161 ± 0.016	0.244 ± 0.050*	0.0203 ± 0.099**	0.03937 ± 0.002
Cerebellum	0.132 ± 0.051	0.019 ± 0.038*	0.083 ± 0.011	0.0933 ± 0.0070

* P < .05

** P < .01

*** P < .001

*When control rats were compares with ovariectomized rats.

Table – 2: Effect Of Estradiol Treatment To Ovariectomized Rat In 5ht Level (µg/Gm Of Tissue)

Brain Regions	Control (Normal Rats)	10 th day after Ovariectomy	Estradiol Treated 24 hrs after(ovariectomized)	Estradiol Treated 36 hrs after ovariectomized)
Hypothalamus	1.342 ± 0.236	0.946 ± 0.098	0.844 ± 0.08**†	0.989 ± 0.343
Striatum	1.086 ± 0.119	0.895 ± 0.226	0.941 ± 0.091*	0.890 ± 0.083
Mid Brain	0.340 ± 0.126	0.288 ± 0.046	0.351 ± 0.04	0.331 ± 0.059
Pons Medulla	1.461 ± 0.016	0.865 ± 0.050	0.717 ± 0.059	0.961 ± 0.340
Cerebral- Cortex	0.161 ± 0.016	0.0203 ± 0.099	0.11 ± 0.172	0.141 ± 0.017
Cerebellum	0.132 ± 0.051	0.083 ± 0.011	0.092 ± 0.38	0.981 ± 0.030

* P < .05

** P < .01

*** P < .001

* When 10 day ovariectomized rats compared with 36hrs estradiol treated rats † When control rats compared with Aa 24hrs estradiol treated rats.

Table – 3: Effect Of Ovariectomy On 5hiaa (µg/Gm Of Tissue) Levels In Different Brain Regions Of Female Rats

Brain Regions	Control (Normal Rats)	5 th day after Ovariectomy	10 th day after Ovariectomy	15 th day after Ovariectomy
Hypothalamus	0.8417 ± 0.18	0.6109 ± 0.082	0.5076 ± 0.0219*	0.5071 ± 0.0225**
Striatum	0.6592 ± 0.19	0.7966 ± 0.035*	0.6494 ± 0.010	0.6816 ± 0.07
Mid Brain	0.1343 ± 0.14	0.01162 ± 0.0253*	0.094 ± 0.0070	0.111 ± 0.0169
Pons Medulla	1.1739 ± 0.04	0.6539 ± 0.056	0.588 ± 0.007*	0.597 ± 0.0014
Cerebral- Cortex	0.1139 ± 0.022	0.1435 ± 0.0070	0.03937 ± 0.002	0.02 ± 0.0141*
Cerebellum	0.1348 ± 0.026	0.1398 ± 0.0070	0.0933 ± 0.008	0.0696 ± 0.0184*

* P < .05

** P < .01

*** P < .001

*When control rats were compared with ovariectomized rats.

Table – 4: Effect Of Estradiol Treatment In 5hiaa Level In Ovariectomized RAT ($\mu\text{g}/\text{gm}$ of tissue)

Brain Regions	Control (Normal Rats)	10 th day after Ovariectomy	Estradiol Treated 24 hrs after(ovariectomized)	Estradiol Treated 36 hrs after ovariectomized)
Hypothalamus	0.8417 \pm 0.18	0.946 \pm 0.098	0.522 \pm 0.001†	0.644 \pm 0.002
Striatum	0.6592 \pm 0.19	0.895 \pm 0.226	0.672 \pm 0.0834	0.862 \pm 0.036
Mid Brain	0.1343 \pm 0.14	0.288 \pm 0.046	0.1112 \pm 0.0172	0.212 \pm 0.004
Pons Medulla	1.01739 \pm 0.04	0.588 \pm 0.050	0.328 \pm 0.0063	0.428 \pm 0.032
Cerebral- Cortex	0.1139 \pm 0.022	0.0203 \pm 0.099	0.1540 \pm 0.079**	0.112 \pm 0.012
Cerebellum	0.1348 \pm 0.026	0.083 \pm 0.011	0.06697 \pm 0.018*	0.09 \pm 0.034

* P < .05

** P < .01

*** P < .001

* When 10 day ovariectomized rats compared with 24hrs estradiol treated rats† When control rats compared with 36hrs estradiol treated rats.

V. Discussion

Estradiol administration increases the production of neurotransmitters like serotonin. It also increases the number of serotonin receptors in hypothalamus¹⁶ (Coope, J (1996) Estradiol exerts significant positive effects on the viability and survival of cortical neurons. Cultured neurons exposed components of CEE and equilin and 17 Beta estradiol have shown a similar effect by enhancing neuronal survival, estradiol preserves the information processing capability and storage capacity for brain cell. The effect of estradiol was to reduce the activity of monoamine oxidase, there is not a likely explanation for the increase in serotonin content since concentration of 5HIAA was increased proportionately as evidenced by the constant ration of 5HIAA concentration to that of serotonin. A more plausible explanation would be a stimulation of tryptophan hydroxylase by estradiol the effects of other hormones on this enzymes activity are not conclusive¹³(Kizer et al 1973) changes in serotonin contents are seen in the dorsal raphe nucleus, the major site of serotonergic cell bodies and also with substantia nigra a region known to contain mainly terminals¹¹ (parent et al 1981) which drives in major part from the dorsal raphe nucleus¹⁴ (Fibiger and Miller 1977). Other regions of fore brain and hind brain, known to receive innervations from the dorsal raphe nucleus were not affected by estradiol treatment with injection of 5 $\mu\text{g}/\text{rat}$ to ovariectomized rat (Crowley et al 1979) observed. No changes in several nuclei of forebrain rostral and medial hypothalamus and midbrain tegmentum. However they did not assay the dorsal raphe nucleus the major site of serotonin cell nor did they investigate the substantia nigra with a similar estrogen administration schedule as Crowley et al (1979)¹² namely one estradiol benzoate 20 $\mu\text{g}/\text{kg}$ to ovariectomized rats 53 hrs before killing¹⁷ (Cone et al 1981) has also observed no changes in serotonin levels in several brain areas except the raphe. With an acute injection of 17 β estradiol at a different dose and time interval between injection of 17 β estradiol at a different dose and time an increase in serotonin levels in the raphe while the other region are unaffected. Cone et al (1981) reported that there was increased in 5HT level after the administration of estrogen along with progesterone to ovariectomized rats in some regions. So therefore estrogen can be effectively administered as an anti depressive agent after further studies.

VI. Conclusion

In this study the levels of serotonin (5HT) and its major metabolic 5HIAA was measured. It was observed that the level of 5HT was decreased in hypothalamus, striatum, mid brain and cerebral cortex but not equally in 5 days, 10 days and 15 days after ovariectomized rats. 5HIAA levels in hypothalamus and cerebral cortex was decreased significantly after 15 days of ovariectomy. In estradiol treated ovariectomized rats, 5HT and 5HIAA level was increased in hypothalamus striatum and pons medulla.

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