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Effect of Acute and Chronic 17β-Estradiol Treatment on Serotonin and 5-Hydroxyindole Acetic Acid Content of Discrete Brain Nuclei of Ovariectomized Rat

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Summary. Increased serotonin content of the dorsal raphe nucleus and of the substantia nigra were seen following acute (12 h or 24 h) administration of 17β-estradiol to ovariectomized rats. No changes were seen in numerous other nuclei of forebrain or hindbrain, and no changes occurred during chronic (1 month) estradiol administration. Changes in the concentration of the major serotonin metabolite, 5-hydroxyindole acetic acid, paralleled those of serotonin. These results show that, under precisely defined conditions and in specific locations only, estradiol can modulate serotonergic neuronal systems.

Key words: Serotonin – Estradiol – Brain nuclei – Raphe – Hydroxyindole acetic acid

Introduction

Serotonin containing neuronal systems are involved in both the control of gonadotropin secretion and mating behaviour (Everitt et al. 1975; Hery et al. 1976; Meyerson and Lewander 1970; Zemlan et al. 1973), functions which are also regulated by ovarian steroids. Changes in brain serotonin have been noted during physiological modulation of gonadal hormones including the estrous cycle and pregnancy (Fludder and Tonge 1975; Greengrass and Tonge 1971, 1974) and serotonin receptor concentration in basal forebrain fluctuates during the estrous cycle (Biegon et al. 1980). Although estradiol injection has been clearly demonstrated to modulate brain serotonin receptors (Biegon and McEwen 1982), the same cannot be said for the serotonin content or metabolism in discrete brain areas. Thus, whilst some authors claim that estrogen administration to ovariectomized animals results in no change in serotonin (Crowley et al. 1979; Bapna et al. 1971; Endersby and Wilson 1974), others indicate an increase in serotonin content (Cone et al. 1981) whilst yet others claim a decrease (Munaro 1978). Reasons for such discrepancies may be found in differences of dose and time of steroid administration, the use of estradiol alone or in combination with progesterone, varying techniques of tissue dissection and an emphasis on the hypophysiotropic area, when it is now apparent that estradiol target cells are also concentrated in numerous structures of the extrahypothalamic brain (Stumpf 1980). We have attempted to clarify this problem by measuring serotonin and its major metabolite, 5-hydroxyindole acetic acid (5–HIAA), in microdissected discrete nuclei of hypothalamic and extrahypothalamic brain at different times after estradiol injection or after chronic treatment with this ovarian hormone alone.

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Materials and Methods

Animals

Female Sprague-Dawley rats (200–250 g) were bilaterally ovariectomized under ether anesthesia upon arrival and maintained in light (lights off 19.00–05.00) and temperature-controlled housing for 1 month before killing. Immediately following ovariectomy, one group was maintained on 17β-estradiol (2 μg/day) in 0.9% NaCl containing 0.3% – gelatin injected s.c. for 4 weeks; their last steroid injection was 24 h before killing. Two further groups of animals were injected with 17β-estradiol (50 μg) at, respectively, 12 h and 24 h before killing, whilst controls were injected with vehicle alone. The dose of estradiol given in chronic studies was the minimum and in acute studies the maximum, previously shown necessary for effects on serotonin receptors in brain (Biegon and McEwen 1982). All animals were killed with a minimum of stress, by decapitation, on the same day between 08.00–09.00 h. Brains were immediately removed and frozen in crushed dry ice.

Table 1. Effects of 17β-estradiol administration on brain serotonin concentrations

Brain region[a]	Serotonin (ng/mg protein)[b] 17β-estradiol			
	Control	12 h	24 h	Chronic
cpm	5.69±0.21	6.43±1.30	7.30±0.90	5.71±0.83
cpl	4.79±0.25	3.92±0.21	5.93±0.39	5.25±0.60
st	8.63±0.85	8.91±0.80	7.06±0.28	6.72±1.28
ha	10.43±0.46	10.56±0.53	9.35±0.52	8.49±1.88
hl	13.65±1.15	12.40±1.25	13.08±0.79	13.48±1.32
am	8.34±0.88	8.44±1.48	9.42±0.80	8.95±0.47
ac	9.37±0.06	7.41±1.49	10.41±0.82	9.57±1.44
tmm	11.06±0.33	9.67±0.95	14.84±2.63	11.94±1.73
hvm	12.57±0.40	11.76±1.54	11.72±1.92	11.06±0.85
hd	15.55±1.69	13.92±0.92	13.26±1.93	15.17±0.54
HI	3.25±0.18	2.83±0.37	3.04±0.16	4.01±0.39
AVT	15.24±0.92	10.95±2.38	19.96±4.05	16.26±1.91
SNR	23.02±2.50	23.62±3.16	33.13±4.73*[c]	22.51±0.73
ip	15.02±2.14	9.69±0.48	12.42±2.01	11.45±0.77
mr	17.61±2.12	23.40±3.20	19.88±1.56	18.57±1.01
dr	24.57±1.97	31.30±1.06*	35.05±4.44*	25.37±4.53
rtp	5.74±0.46	5.06±0.40	6.91±0.60	8.52±3.50
rm	9.29±1.53	8.79±0.58	11.56±1.45	7.25±2.11
rp	7.33±0.97	8.54±1.09	11.31±1.15	9.00±1.65
nts	16.56±2.94	14.61±1.46	17.14±1.38	13.95±1.72

[a] Abbreviations used: cpm, nucleus (n) caudatus putamen (median); cpl, n. caudatus putamen (lateral); st, n. interstitialis striae terminalis; ha, n. anterior (hypothalami); hl, n. lateralis (hypothalami); am, n. amygdaloideus medialis; ac, n. amygdaloideus centralis; tmm, n. medialis thalami; hvm, n. ventromedialis (hypothalami); hd, n. dorsalis (hypothalami); HI, hippocampus; AVT, area ventralis tegmenti; SNR, substantia nigra, zona reticulata; ip, n. interpeduncularis; mr, n. medianus raphes; dr, n. dorsalis raphes; rtp, n. reticularis tegmenti pontis; rm, n. raphe magnus; rp, n. raphe pallidus; nts, n. tractus solitarii

[b] Tissue was pooled from 2 animals and the results shown are the mean ± SEM of 4 such groups

[c] The statistical difference between means was tested using the multiple range test of Kramer following analysis of variance

* = $p < 0.05$

Table 2. Effects of 17β-estradiol administration on 5-HIAA concentrations of brain nuclei

Brain area[a]	[5-HIAA] / [Serotonin] 17β-estradiol			
	Control	12 h	24 h	Chronic
cpm	1.34±0.13	1.03±0.21	1.12±0.16	1.38±0.22
cpl	1.57±0.10	1.63±0.09	1.41±0.14	1.40±0.19
st	1.08±0.13	1.07±0.10	1.24±0.09	1.17±0.23
ha	0.55±0.04	0.54±0.03	0.65±0.07	0.65±0.16
hl	0.66±1.06	0.73±0.08	0.75±0.08	0.66±0.08
am	0.63±0.08	0.73±0.15	0.75±0.07	0.73±0.09
ac	0.91±0.03	1.33±0.35	1.07±0.15	0.95±0.18
tmm	0.87±0.03	1.09±0.12	0.86±0.16	0.89±0.13
hvm	0.65±0.02	0.57±0.09	0.66±0.11	0.56±0.06
hd	0.56±0.10	0.83±0.06	0.84±0.12	0.71±0.04
HI	1.14±0.09	1.95±0.52	1.29±0.09	1.33±0.10
AVT	0.75±0.05	0.97±0.26	0.75±0.17	0.76±0.19
SNR	0.56±0.06	0.63±0.10	0.58±0.13	0.63±0.05
ip	1.29±0.21	1.39±0.13	1.24±0.26	1.31±0.16
mr	1.53±0.24	1.49±0.21	1.59±0.17	1.36±0.08
dr	1.05±0.11	0.94±0.05	0.97±0.14	1.07±0.19
rtp	2.26±0.32	2.49±0.23	2.21±0.28	2.11±1.32
rm	0.74±0.13	0.63±0.05	0.64±0.08	0.68±0.24
rp	1.66±0.22	1.61±0.26	1.62±0.29	1.54±0.32
nts	1.43±0.30	1.62±0.26	1.48±0.14	1.28±0.28

[a] Abbreviations used are listed in the legend to Table 1

Tissue Extraction

Slices of brain 300 μm thick were cut in the coronal plane in a freezing microtome at –10° C. Specific brain nuclei were removed using stainless steel punches of 0.5 mm or 1.0 mm internal diameter according to Palkovits (1973). Following homogenization in 100 μl of 0.1 N perchloric acid and centrifugation at 10,000 g for 20 min, the supernatant was removed for serotonin assay and the protein content of the pellet measured (Lowry et al. 1951).

Serotonin and 5-Hydroxyindole Acetic Acid Assay

Supernatant (80 μl) was injected into a Beckman liquid chromatograph (chromegabond MC-18 column) equipped with a model LC-4 electrochemical detector (Bioanalytical systems, Indiana, USA) and CP-O carbon paste electrode. The mobile phase was 0.1 M $CH_2ClCOOH$, pH 3.0 containing 15% methanol and delivered at a flow rate of 2.0 ml/min. The electrochemical detector electrode potential was set at 0.65 V with respect to a $Ag^+/AgCl$ reference electrode. Serotonin and 5-HIAA concentrations were determined from peak heights and by comparing them to authentic standards (Sigma, St. Louis, Mo.) subjected to the identical procedure. All concentrations are expressed as the free-base, and a linear relationship of peak height to concentration was obtained over the range of 0.02 ng to 5 ng of serotonin or 5-HIAA.

Results

The serotonin concentrations of 20 different forebrain, midbrain and hindbrain nuclei are shown in Table 1. Highest concentrations were seen in the dorsal raphe nucleus, closely followed by substantia nigra, median raphe nucleus and the nucleus of the solitary tract in agreement with previous findings (Saavedra 1977). After 12 h following injection of 17β-estradiol into ovariectomized rats, the serotonin content of the dorsal raphe nucleus was significantly increased by 27%. This increase was maintained at 24 h following injection and at this time an increase in serotonin content was also noted in the substantia nigra, zona reticulata. No significant differences were evident between the serotonin content of brain nuclei in ovariectomized rats and those of animals chronically treated with 17β-estradiol. Significant increases in the content of 5-HIAA in the dorsal raphe nucleus were seen 12 h and 24 h after estradiol injection and in the substantia nigra 24 h after injection. The ratio of 5-HIAA concentration to that of serotonin, which

may be indicative of neurotransmitter turnover and utilization (Mefford et al. 1982) was not affected by acute or chronic estradiol administration (Table 2).

Discussion

Our findings clearly demonstrate that 17β-estradiol can influence the serotonin concentration of certain brain areas. However, this effect is restricted not only to two specific nuclei, but also by the duration of estradiol administration. Thus, whilst we see changes at 12 h and 24 h after estradiol injection, these modulations are no longer apparent following chronic estradiol administration. Such an effect of acute estradiol administration but not chronic has also been noted on the activity of type A monoamine oxidase in certain nuclei of rat brain stem (Chevillard et al. 1981). Although the effect of estradiol was to reduce the activity of monoamine oxidase, this is not a likely explanation for the increase in serotonin content we describe here, since the concentration of 5-HIAA is increased proportionally as evidenced by the constant ratio of 5-HIAA concentration to that of serotonin. A more plausible explanation would be a stimulation of tryptophan hydroxylase by estradiol, although to our knowledge, no such effect has been reported and the effects of other hormones on this enzyme activity are not conclusive (Kizer et al. 1976; Sze et al. 1976). Changes in serotonin content are seen in the dorsal raphe nucleus, the major site of serotoninergic cell bodies and also in the substantia nigra, a region known to contain mainly terminals (Parent et al. 1981) which derive in major part from the dorsal raphe nucleus (Fibiger and Miller 1977). Other regions of forebrain or hindbrain, known to receive innervation from the dorsal raphe nucleus were not affected by estradiol treatment. With one injection of estradiol benzoate (5 μg/rat) to ovariectomized rats (4 weeks before) and killing 54 h later, Crowley et al. (1979) observed, as we report here, no changes in several nuclei of the forebrain, rostral and medial hypothalamus and midbrain tegmentum. However, in their study, they did not assay the dorsal raphe nucleus, the major site of serotonin cell bodies, nor did they investigate the substantia nigra. With a similar estrogen administration schedule as Crowley et al. (1979), namely one estradiol benzoate injection (20 μg/kg) to ovariectomized rats (4–8 weeks previously) 53 h before killing, Cone et al. (1981) have also observed no changes in serotonin levels in several brain areas except the raphe. With an acute injection of 17β-estradiol at a different dose and time interval between injection and killing, we observe as Cone et al. (1981) an increase in serotonin levels in the raphe while the other regions are unaffected and the substantia nigra was not investigated in their study. The rate of serotonin accumulation after inhibition of degradation with pargyline was used by Cone et al. (1981) to reflect synthesis of the neurotransmitter. Serotonin accumulation was increased after estradiol plus progesterone treatment of ovariectomized rats in the dorsal raphe while no changes were observed in the other brain regions investigated (Cone et al. 1981). These results are in agreement with our observation of increased levels of serotonin and 5-HIAA in the dorsal raphe of estradiol treated rats, suggesting that the changes observed are from a stimulation of synthesis rather than a decreased turnover or utilization. Thus, the acute effect of estradiol on brain serotonin levels is observed at different doses (5–50 μg/rat) and time after injections (12–54 h) in rats deprived of ovarian steroids for 1 to 4 weeks. By contrast, after a chronic treatment with this steroid (2 μg/day/rat) for 1 month and killing 24 h after the last injection, no changes are observed. Although steroid hormones have been implicated in the control of many central nervous system functions (McEwen et al. 1979), it is difficult at present to comment on the relevance of our findings to the postulated roles for serotonin in the brain (Chase and Murphy 1973).

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