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Effects of Olfactory Bulbectomy and Estrogen on Tyrosine Hydroxylase and Glutamic Acid Decarboxylase in the Nigrostriatal and Mesolimbic Dopamine Systems of Adult Female Rats[1]

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TYLER, J. L., J. H. GORDON AND R. A. GORSKI. *Effects of olfactory bulbectomy and estrogen on tyrosine hydroxylase and glutamic acid decarboxylase in the nigrostriatal and mesolimbic dopamine systems of adult female rats.* PHARMAC. BIOCHEM. BEHAV. **11**(5) 549–552, 1979.—Following olfactory bulbectomy (BULBX), ovariectomized female rats show enhanced behavioral sensitivity to estradiol benzoate (EB) as measured by an index of sexual receptivity, lordosis responding. We have proposed previously that alterations in EB sensitivity which also are produced by septal destruction reflect disruptions of gamma-aminobutyric acid (GABA) inhibitory feedback on dopamine (DA) cell bodies in the midbrain, which may be inhibitory to the expression of lordosis. Since the olfactory system as well as the septum receives mesolimbic DA projections, in the present study we examined the effects of EB given to bulbectomized and sham-operated rats on tyrosine hydroxylase (TH) activity in the olfactory tubercle (OT), nucleus accumbens septi and corpus striatum. Glutamic acid decarboxylase (GAD) activity was measured in the ventral tegmental region (VTR) and the substantia nigra (SN). Rats received 2 μg EB/day for 3 days and were tested on Day 4 in a 20 mount behavioral test. Ten bulbectomized rats demonstrating enhanced behavioral sensitivity to EB and all sham-operated rats (N = 10) were selected for further study. Five rats in each group received 0.05 ml oil/day X 3 (BULBX-oil, SHAM-oil), and five received 2 μg EB/day X 3 (BULBX-EB, SHAM-EB). Rats were sacrificed on Day 4. Subsequent assays revealed a bulbectomy-dependent decrease in TH activity in the striatum, and a lesion plus EB-dependent decrease in TH activity in the OT. GAD activity was slightly but significantly suppressed in the VTR in the SHAM-EB group relative to that in the SHAM-oil group. Rats of the BULBX-EB group failed to exhibit decreased GAD activity. Thus, bulbectomy may result in enhanced behavioral sensitivity to EB due to disruptions in GABA-DA interactions, which are similar to those observed following septal destruction and which result in diminished behaviorally inhibitory DA tone.

Olfactory bulbectomy Dopamine GABA TH activity GAD activity Olfactory tubercle
Lordosis EB Limbic system

OLFACTORY bulbectomy (BULBX) in the female rat results in a dose-dependent increase in sensitivity to estradiol benzoate (EB) as measured by sexual receptivity [3, 16, 27]. Septal lesions produce a similar effect [8, 18, 19]. Recently, changes in the regulation of dopamine (DA), which is reputedly inhibitory to the expression of lordosis [1,4], and gamma-aminobutyric acid (GABA) were reported following EB administration in septal lesioned rats [8,9]. Since the olfactory tubercle (OT) is a major projection site of bulbar efferents, as well as mesolimbic DA efferents, we tested the hypothesis that altered GABA-DA interactions in response to EB might accompany the enhanced behavioral sensitivity induced by bulbectomy. We examined the effects of bulbectomy on tyrosine hydroxylase (TH, the rate-limiting enzyme

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in DA synthesis) and glutamic acid decarboxylase (GAD, the rate-limiting enzyme in GABA synthesis) activities in response to EB. The nucleus accumbens (N.Acc) and corpus striatum (CST) were analyzed in addition to the OT for TH activity, since these regions contain mesolimbic and nigrostriatal DA terminals. GAD activity was measured in the substantia nigra (SN) and ventral tegmental region (VTR), since these are regions to which axons of GABA cells apparently project and are also sites of DA cell bodies.

METHOD

Adult female Sprague-Dawley rats were purchased from Simonsen Laboratories (Gilroy, CA). Rats were housed in individual cages with Purina rat chow and water available ad lib. They were maintained on a reversed 13:11 light/dark schedule (lights on 2300–1200 hr). The animals were ovariectomized under ether anesthesia and two to four weeks later received one of 2 surgical treatments. All surgery was performed under sodium methohexital (Brevital) anesthesia. Bilateral BULBX (N=20) was produced by lowering a knife anterior to the junction of the bulbs with the frontal cortex. Tissue anterior to the cut was aspirated, and the cavity was filled with oxycel for homeostasis. Sham surgery (SHAM, N=10) consisted of exposing the dura over the olfactory bulbs.

Behavioral Testing

Although rats received ovarian steroids and behavioral tests as part of preliminary studies, no steroids were administered for ten days prior to the start of this behavioral test. The rats received a subcutaneous injection of 5 μg EB/Kg daily for 3 days. On Day 4 reproductive behavior was tested. At 1400–1500 hr, each rat was placed in a Plexiglas arena containing 2–3 previously adapted sexually vigorous Long-Evans male rats. Behavioral responses were recorded until 20 mounts had occurred. The lordosis quotient (LQ) was calculated: LQ=number of lordotic responses × 100 divided by 20 mounts.

Biochemical Studies

Two weeks later, half of the 10 BULBX rats which had shown elevated LQ scores and half of the SHAM group were given oil (0.05 ml) sc for 3 days, and the other half of each group was given 5 μg EB/Kg/day for 3 days. On Day 4, at 1400–1500 hr, the rats were sacrificed by decapitation. In less than 1 min, brains were removed, rinsed in cold saline, and frozen on solid CO_2. Frozen brains from rats in the BULBX group were viewed from ventral, dorsal and lateral perspectives. The extent of bulb aspiration was recorded. The frozen brains were wrapped in aluminum foil and then stored at −50°C prior to dissection and assay of TH and GAD activity.

Dissection of Brain Tissues

Frozen brains were placed on chilled aluminum blocks in an ice bath and thawed slightly. Three frontal cuts were made perpendicular to the ventral surface of the brain at the level of the rostral, middle and posterior borders of the olfactory tubercle. Tissue rostral to the first cut was discarded. From the remaining two sections, bilateral samples of OT were dissected. Bilateral NAcc tissue was dissected from the first section, and one CST was dissected from the second section. Additional frontal cuts were made to isolate a section of tissue 1 to 2 mm thick immediately posterior to the mammillary bodies. The VTR and SN samples were dissected from this section. Bilateral samples were pooled.

Biochemistry: Homogenate Preparation

Homogenate samples for TH and GAD assays were prepared in the same manner. Tissue samples, 3 to 15 mg, were homogenized in 50 μl of 0.005 M Tris buffer (pH=7.0) and then again after the addition of 25 μl of 1.5% Triton × 100. A 50 μl homogenate sample was removed for Lowry's [15] protein analysis, using bovine albumin for the standard. Duplicate 25 μl homogenate samples were placed in 12×75 mm culture tubes in an ice bath, and reaction mixture was added.

GAD Assay

VTR and SN samples were assayed for GAD activity using the methods of Gordon *et al.* [8]. Reaction mixture (100 μl/tube) was added to make final concentrations in each tube of 80 nM potassium phosphate (pH=7.0), dl-1-^{14}C glutamic acid (4.0 nM, 0.1 μCi/Mole), and pyroxidol phosphate (0.5 μM). The conversion of ^{14}C glutamate to GABA by GAD produces $^{14}CO_2$, which was used to determine the amount of GAD activity. GAD activity, inferred from $^{14}CO_2$ levels, was defined in terms of nMoles GABA formed/mg protein/hour. The assay was conducted with duplicate samples. Hyamine hydroxide saturated paper (3/4 in. square) was placed in the tubes during incubation to trap $^{14}CO_2$; the paper was then treated with scintillation fluor and sample $^{14}CO_2$ levels were counted in a Beckman spectrometer.

TH Assay

Homogenate samples from OT, CST and NAcc were assayed for TH activity using the methods of Levitt *et al.* [13] with modifications described by Mueller *et al.* [17]. The reaction was carried out at 37°C for 30 min in a final volume of 50 μl with a substrate concentration of 200 μM 1-^{3}H (2,5)-tyrosine (0.1 μCi/μMole) and cofactor concentration of 600 μM (5,6 dimethyl-5,6,7,8-tetrahydropterine·HCl). The assay was conducted with duplicate samples. The conversion of ^{3}H-tyrosine to DOPA by TH is a stoichiometric reaction which produces $^{3}H_2O$, which was measured after purification by ion exchange chromatography and used to determine the amount of TH activity. TH activity was defined in terms of pmols DOPA formed/hour/mg protein.

Statistics

Statistical comparisons were made by Student's *t*-test [2] and the Spearman rank coefficient [25]

RESULTS

Analysis of the aspiration boundaries in the brains of the BULBX group animals revealed minimal damage to the frontal cortex in one animal. In no case did the caudal extent of the aspiration intrude on the olfactory peduncle. Varying amounts of main bulb tissue were removed. The accessory bulbs were not always removed, but the vomeronasal nerves were sectioned.

The mean LQ ± standard error of the BULBX group was 82.5 ± 6.1, and the SHAM group was 30 ± 9.3 ($p<0.001$,

TABLE 1

THE INFLUENCE OF ESTRADIOL BENZOATE (EB) TREATMENT OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN SHAM OPERATED AND OLFACTORY BULBECTOMIZED (BULBX) FEMALE RATS

	SHAM		BULBX	
	oil*	EB†	oil*	EB†
Ventral Tegmental Region	47.87 ± 1.16‡	43.34 ± 0.68§	48.34 ± 4.40	47.46 ± 4.85
Substantia Nigra	149.00 ± 8.10‡	140.18 ± 5.80	147.59 ± 12.80	130.99 ± 10.90

*0.05 ml oil given for 3 days.
†2 μg EB in 0.05 ml oil given for 3 days.
‡Mean + standard error expressed as nmoles GABA formed per mg protein per hr.
§Significant decrease from oil-treated SHAM group values ($p<0.02$, 2-tailed t test).

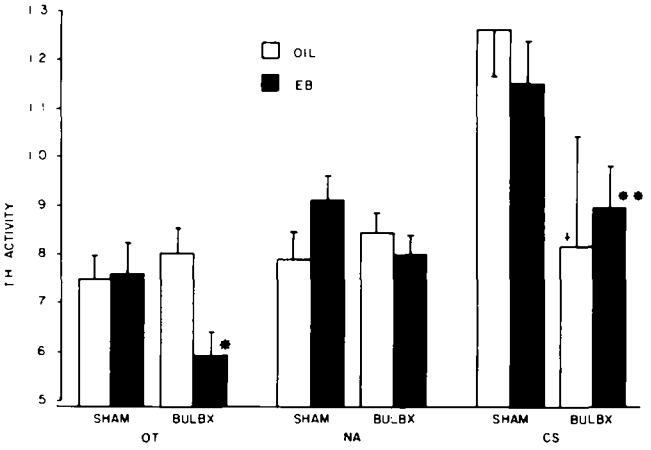

FIG. 1. Influence of estradiol benzoate (EB) treatment on tyrosine hydroxylase (TH) activity (expressed as pmoles DOPA formed/mg protein/hr) in the olfactory tubercle (OT), nucleus accubens (NA) and corpus striatum (CS) in sham operated (SHAM) and olfactory bulbectomized (BULBX) female rats. *Significant decrease from both oil treated group ($p<0.05$, two-tailed t-test). **Significant decrease from both SHAM (oil or EB) group values ($p<0.05$, one-tailed t-test). †Significant decrease from oil-treated SHAM group values ($p<0.05$, one-tailed t-test). Standard error of the mean indicated at the top of the bars.

Mann-Whitney U test). BULBX group rats were ranked on the basis of the amount of bulb tissue removed. The correlation between extent of bulbectomy and subsequent LQ was not significant (Spearman's rho = −0.298). Each surgical treatment group was divided into two separate groups, balanced for body weight and LQ, for testing TH and GAD activity. Thus, there were no differences in LQ or weight between BULBX (or SHAM) group rats which subsequently received oil and those which received EB, prior to sacrifice and biochemical analysis.

TH activity in the OT, NAcc and CST are presented in Fig. 1. Comparable to previous studies [8], EB did not alter TH activity in the sham-operated rats in the OT, NAcc or CST. TH activity in the CST of the BULBX group rats was less than that of the SHAM group rats ($p<0.05$, one-tailed). Administration of EB did not alter striatal TH activity, and BULBX rats maintained lower levels than did either EB or oil treated SHAM rats ($p<0.05$). EB significantly depressed TH activity in the OT of the BULBX group rats relative to all other groups ($p<0.05$). The level of GAD activity in the VTR (Table 1) was slightly but significantly depressed by EB in the SHAM ($p<0.02$), but not in the BULBX group rats. GAD activity in the SN tended to be depressed by EB, but the degree of suppression did not reach significance.

DISCUSSION

The present results demonstrate that one response to bulbectomy is decreased TH activity in the CST; EB-dependent changes in TH and GAD activity were also observed. The biochemical effects of EB in the BULBX group rats are different from those in the SHAM group rats in at least two respects: (1) BULBX group rats respond to EB with a decline in TH activity in the OT, and (2) in BULBX group rats VTR levels of GAD activity fail to decrease following EB. These BULBX-induced changes are similar to those seen in septal-lesioned females [8] and septal-lesioned males sensitized to the acute effects of EB [9]. Furthermore, like septal-lesioned female and sensitized septal-lesioned male rats, bulbectomized rats show enhanced behavioral sensitivity to EB treatment, as measured by the LQ.

Trauma-induced alterations in DA-GABA interactions in response to EB may be part of the neurochemical bases for enhanced behavioral sensitivity to EB following septal lesions [8] or BULBX. It is generally accepted that a gabaminergic striatonigral inhibitory circuit exists [12, 23, 28]. A similar inhibitory GABA-DA feedback circuit involving DA cell bodies in the VTR has been proposed [6]. A decrease in DA concentrations or activity could reduce DA-mediated stimulation of inhibitory GABA activity in the region of the DA cell bodies (SN and VTR). The resultant compensatory decrease in GABA activity could help maintain dopaminergic tone in the nigrostriatal and mesolimbic systems.

It has been proposed that estrogen acts to decrease the postsynaptic efficacy of DA [8,9]. Treating an intact animal with EB could decrease either the number or the affinity of postsynaptic DA receptors, thus decreasing the effective-

ness of released DA. This decrease could in turn decrease the inhibitory feedback of GABA in the area of the DA cell bodies. However, BULBX, as well as septal lesions, appears to interfere with the ability of the GABA neuronal feedback system to compensate for the presence of EB, resulting in a decrease in TH activity. The deficit in the GAD response to EB following BULBX (or septal lesions) supports this concept. This theory is particularly applicable to the effects of BULBX, since both the olfactory bulbs [10,11] and the VTR [5, 14, 26] project to the OT, which is rich in estradiol-concentrating cells [22]. As a result of decreased striatal TH activity and a failure to respond to EB with a compensatory GAD decrease, the bulbectomized, EB-treated rat may have a deficit in dopaminergic tone. Since pharmacological data demonstrate that DA is generally inhibitory to the expression of sexual receptivity [1,4], BULBX-induced alterations in the regulation of GABA-DA interactions may be part of the neurochemical substrate supporting enhanced sexual receptivity in response to EB.

Plasticity in neural organization [7,24] and function [9, 20, 21] induced by surgical trauma is well-documented, but the neurochemical and neuroendocrine correlates of the recovery processes following such trauma are not well understood. Enhanced behavioral sensitivity to EB following BULBX or septal lesions may provide a model system for the investigation of neurochemical mechanisms through which hormonal influences are exerted on neuronal function.

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