Estradiol benzoate, norepinephrine, and weight regulatory behavior in female rats

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Estradiol benzoate (EB: 0, 10, 25, 50, and 100 micrograms) was administered to prepubertal, pregnant, pseudopregnant, lactating, and ovariectomized female rats. Estradiol decreased feeding and increased locomotion in the ovariectomized rats only. A second experiment examined the eating and activity of these same preparations following intraventricular norepinephrine (NE: 2 micrograms) administration. NE reduced feeding and enhanced activity in all groups. The findings are interpreted to mean that NE mediates estradiol's effect on weight regulatory behavior.

Estradiol influences the food intake and spontaneous activity of female rats. During estrus (vaginal cornification) one observes decreased feeding and heightened running (Kennedy & Mitra, 1963; Rodier, 1971; Stern & Zwick, 1972). Ovariectomy produces an increase in food consumption and a dramatic decrease in activity; estradiol benzoate (EB) reverses these consequences (Mook, Kenney, Roberts, Nussbaum, & Rodier, 1972; Wade & Zucker, 1970a).

In contrast to its effects on intact and ovariectomized rats, EB does not affect the feeding or running of prepubertal (Wade & Zucker, 1970b; Wade, 1975; Porterfield & Stern, 1974), pregnant, pseudopregnant or lactating females (Wade, 1972). The interpretation most frequently encountered for these findings is that the brain systems managing weight regulatory behavior are refractory to estradiol in these preparations. Most (if not all) of the work in this field has employed dosages of EB equal to or less than 10 micrograms. To date, no one has investigated the possibility that the brain systems are not actually refractory but merely exhibiting an elevated threshold to estradiol. The present study tested this possibility by administering a wide range of EB (0-100 micrograms) to these "refractory" animals.

EXPERIMENT I

Method

Animals. The subjects were 75 Sprague-Dawley female rats. The animals were housed individually with standard rat chow and tap water available ad lib. The laboratory's temperature was thermostatically maintained at approximately 72°F with a 12-h on-off light cycle; lights off at 8: p.m.

Apparatus. Activity and food intake were measured in two 24 x 24 x 12 in. enclosed Plexiglas chambers. The chambers were divided into quadrants by photocells. Breaking a beam for $\geq .5 \leq 2.0$ sec was automatically recorded as a crossover on an Esterline Angus event recorder. Following a crossover, the system was refractory for 1 sec. Each chamber had a food bin and water bottle attached to one wall.

Procedure. All animals received 5 days of EB (0, 10, 25, 50, or 100 micrograms/day) stimulation prior to testing. The estradiol,

suspended in sesame oil, was administered subcutaneously (.10 ml) during the 1st h of the lights-on period (approximately 8:00 a.m.). The prepubertal females were treated on Days 25-29 of life. The pregnant and pseudopregnant (induced with 10-mg progesterone administered on the morning following behavioral estrus; Everett, 1963) rats were administered EB on postestrus Days 6-10. Lactating animals were treated on postpartum Days 6-10. Each lactating rat had a litter of six pups; litter size has been shown to influence a mother's general health (Stern & Bronner, 1970). Finally, ovariectomized animals at least 4 months old were administered EB for 5 days, starting 3 to 6 weeks after surgery, and tested.

Immediately following the fifth injection, subjects were placed in the testing apparatus. At lights-out (12 h later), the amount of food in the chamber was weighed and the recording equipment activated. Crossovers and food intake to the nearest .1 g were noted the following morning.

A Kruskal-Wallis one-way analysis of variance by ranks was performed for each group across treatments. Between-group comparisons were made using Mann-Whitney U tests. All p values are based on two-tailed tests.

Results

Activity. Table 1 shows the mean number of crossovers for the females following 5 days of estradiol stimulation. Estradiol, in both physiological and pharmacological dosages, was ineffective in stimulating activity in the prepubertal, pregnant, pseudopregnant, or lactating animals. In contrast, the ovariectomized rats displayed significantly more activity with EB treatment ($p < .001$). There was no difference between the 10-, 25-, 50-, or 100-microgram treatments.

Comparing the groups individually shows the

Table 1
Mean Number of Crossovers for the Females Following
5 Days of EB Stimulation

Condition	Estradiol Benzoate (μ g)				
	0	10	25	50	100
Prepubertal	298	306	343	290	278
Pregnant	350	333	371	401	311
Pseudopregnant	408	384	315	481	442
Lactating	268	300	211	215	372
Ovariectomized	298	403	475	431	501

ovariectomized rats to be the most active. Given that these females were the only ones being stimulated by estradiol, this finding is not too surprising. Of more interest is the fact that the pregnant and pseudopregnant rats consistently displayed more activity than the prepubertal or lactating rats ($p < .01$). The latter two groups did not differ. Finally, the pregnant group displayed fewer crossovers than the pseudopregnant rats ($p < .05$).

Of interest also is the number of live young born to the females receiving estrogenic stimulation. The greater the level of estradiol stimulation, the fewer live young born (Table 2). It must be emphasized, however, that there were only three animals in each group and that these data are most preliminary.

Food intake. Table 3 indicates the food consumption of the females. As was the case with crossovers, EB had no effect on feeding in prepubertal, pregnant, pseudopregnant, or lactating rats. Estradiol did, however, significantly depress eating in the ovariectomized rats ($p < .05$). There was no relationship between EB dosage and the level of feeding suppression.

The lactating and prepubertal rats ate significantly more ($p < .02$) than the pregnant and pseudopregnant rats when food intake was computed over body weight. There was no difference between the prepubertal and lactating groups or between the pregnant and pseudopregnant groups.

EXPERIMENT II

While Experiment I emphasizes the inability of EB to influence activity and feeding in certain preparations, EB does exert a major influence over these behaviors in intact and ovariectomized rats. Three recent studies have suggested that estradiol modulates weight regulatory behavior by altering brain catecholamine levels. Simpson and Dicara (1973) report that anterior hypothalamic injections of NE elicit eating irrespective of circulating estradiol levels. Intracerebral dopamine increases feeding only with low blood estradiol titers. These investigators conclude that estradiol inhibits dopamine hydroxylase activity, thereby reducing brain NE levels and feeding. Stern and Zwick (1973) report that intraventricular EB (1 microgram) and NE (50-150 micrograms) stimulate activity and reduce feeding

Table 2
Number of Live and Stillborn Young Delivered by the Females Following 5 Days of EB Stimulation During Pregnancy

EB (μ g)	Live Young	Stillborn
0	8, 11, 12	0, 0, 0
10	9, 11, 13	0, 0, 0
25	8, 8, 9	0, 0, 0
50	7, 8, 9	2, 0, 0
100	5, 8, 9	2, 1, 0

Table 3
Mean Food Intake (Grams) of the Females Following 5 Days of EB Stimulation

Condition	0	Estradiol Benzoate (μ g)			
		10	25	50	100
Prepubertal	12.5	13.2	11.8	12.2	11.0
Pregnant	15.3	16.1	14.5	14.2	15.8
Pseudopregnant	14.5	13.6	15.5	14.8	15.3
Lactating	36.5	34.5	32.3	37.8	35.5
Ovariectomized	16.2	12.5	11.6	11.9	10.0

when administered in the dark. Pretreatment with phentolamine, an α -adrenergic receptor antagonist, prevented the activity increase associated with both treatments; imipramine, an adrenergic agonist, potentiated the behavioral effects of EB and NE. Stern and Zwick propose that estradiol increases levels of functional brain NE, thereby stimulating running and decreasing feeding. Finally, EB depresses eating and stimulates activity less in ovariectomized rats deprived of rapid eye movement sleep (REM) than in controls (Longuski, Cudillo, & Stern, submitted for publication). On the assumption that REM sleep helps maintain catecholamine functioning in the brain (Stern & Morgane, 1974), the data were interpreted to mean that estradiol influences weight regulatory behavior through brain catecholamines.

The present study examines the effects of intraventricular NE in prepubertal, pregnant, pseudopregnant, and lactating rats. If, as suggested above, NE mediates estradiol's effects on activity and feeding, it was reasoned that NE might directly stimulate the estradiol-sensitive neurons in these preparation (i.e., bypass the estradiol refractoriness) and produce weight regulatory changes.

Method

Procedure. The maintenance of the subjects was identical to that in the previous study. All subjects had cannula guides implanted stereotactically to a point 1 mm above the right lateral ventricle (DeGroot: A 5.4, L 2.2, V -4.0). The coordinates used for the prepubertal animals (21-25 days old at implantation) were "corrected" for the difference in size between the brain of the adult and that of the juvenile rat (A .9, L 1.6, V -3.2). The guide assemblies consisted of an outer shaft made of 22-ga. stainless steel tubing and an inner removable cannula of 28-ga. tubing. The outer shafts were permanently attached to the skull with machine screws and dental acrylic resin. Surgery was conducted under pentobarbital anesthesia.

Testing. At lights-out, 3-11 days after implantation, the animals were placed in wire holding cages that prevented movement. The inner cannula was removed, and a needle was inserted 1 mm below the guide shaft. Evidence that the ventricle had been penetrated was provided by the spontaneous leakage of cerebrospinal fluid (CSF) or by subsequent histological examination (see below). The needles used to introduce the agents were attached to one of two microliter syringes. The agents, .154 M NaCl or 1.625 mM (2 micrograms) norepinephrine (L-arterol bitartrate monohydrate, Sigma) made isotonic to CSF (.154 M) with NaCl, were infused over a 15-sec period in 10 microliters fluid. Immediately after infusion, the animals were placed in testing chambers.

The pregnant and pseudopregnant (see Experiment I) rats were

tested on postestrus Days 7 and 11. Immature rats were tested on Days 30 and 34 of life. Lactating animals were tested on postpartum Days 7 and 11; again, the lactating females each had a litter of six pups. The ovariectomized rats, tested at least 2 weeks after gonadectomy, received two tests separated by 96 h.

Crossovers and food intake were recorded 12 h after infusion.

Histology. The females were sacrificed with an overdose of sodium pentobarbital and perfused pericardially with .9% sodium chloride and neutralized 10% Formalin. The removed brains were fixed in Formalin. Frozen sections cut at 50 micrometers were stained with cresyl violet and examined microscopically to determine cannula placements. Only those animals with placements superior to the ventricle (82%) are considered in the data analysis.

Results

Table 4 shows the mean number of crossovers and food intake for the females during testing. In all conditions, NE (2 micrograms) had a significant effect on both activity and eating: NE infused intraventricularly increased activity and limited feeding in prepubertal, pregnant, pseudopregnant, and lactating rats.

It is noteworthy that only two rats (one pregnant and one lactating) displayed bouts of retching or vomiting following infusion. This is in contrast to the 15% of the animals (25% of the tests) that evidenced such behavior in Stern & Zwick's (1973) report.

DISCUSSION

The present results clearly show that in prepubertal, pregnant, pseudopregnant, and lactating rats, the brain systems controlling activity and feeding are refractory to EB. Estradiol influenced crossovers and food intake in ovariectomized rats only (Tables 1 and 3). In each case, however, the underlying reasons for the refractoriness appear varied.

The ventromedial hypothalamus (VMH) is a region of major importance in estradiol's effect on feeding. Implants of crystalline EB in this region limit food intake in adult females (Wade & Zucker, 1970a; Jankowiak & Stern, 1974). VMH lesions in prepubertal rats do not produce the increase in feeding that is observed in adults, suggesting that the VMH does not restrain feeding in the immature rat (Bernardis, 1966; Kennedy, 1957). Kennedy (1967) has proposed that the VMH is sensitive to a correlate of body fat and that the weanling is too lean to activate the region. Perhaps, then, estradiol does not limit eating in juvenile rats because its major effect is through a behaviorally inactive brain region.

The ineffectiveness of EB on activity in immature rats has been studied by Porterfield and Stern (1974). EB (10 micrograms) was administered to hypophysectomized and ovariectomized rats starting at 25 days of age. Estradiol significantly increased activity as measured in revolving drums. If Bovine growth hormone (1 mg) was administered with the EB, the increase in activity was not observed. It was concluded

Table 4
Mean Number of Crossovers and Food Intake (g) Following Intraventricular Infusion (10 μ l) of Saline or NE (2 μ g)

Condition	Saline		Norepinephrine (2 μ g)	
	Crossovers	FI	Crossovers	FI
Prepubertal (n = 9)	265	12.5	461*	10.4*
Pregnant (n = 17)	350	15.3	543*	12.4*
Pseudopregnant (n = 14)	408	14.5	673*	13.1*
Lactating (n = 11)	245	38.5	365*	33.6*
Ovariectomized (n = 6)	215	14.1	440*	11.5*

*Significant ($p < .05$) difference from saline controls

that growth hormone masks the actions of estradiol on activity in weanlings. The inability of growth hormone to block estradiol's effects in adult animals awaits clarification.

During pregnancy, rats show elevated plasma progesterone (Hashimoto, Henricks, Anderson, & Melampy, 1968). Antiestrogenic actions for progesterone are well established in peripheral tissues and estrous behavior (Courrier, 1951; Powers & Zucker, 1969). Also, it has been suggested that progesterone inhibits the uptake of tritiated estradiol by rat brain (Anderson & Greenwald, 1969). The inability of exogenous EB to influence activity or feeding in these preparations, therefore, may be caused by the high levels of endogenous progesterone preventing EB from expressing itself. For some time now, Wade (1972) has maintained that estradiol is the principle ovarian steroid controlling energy regulation and that progesterone merely blocks its effects. The present data are clearly consistent with this view.

A number of factors contribute to the inability of estradiol to affect energy regulation in lactating rats. Firstly, Kennedy (1953) has shown that VMH lesions do not produce hyperphagia in lactating animals. As was the case in weanlings, the VMH appears behaviorally inactive. It has been suggested that the large energy drain related to milk production masks hypothalamic restraint of feeding (Wade, in press). Plasma progesterone levels are elevated during nursing and, as already discussed, progesterone blocks the uptake of estradiol by the brain. Lastly, prolactin may also prevent the actions of estradiol on neural tissue. Estradiol treatments that inhibit prolactin release also depress feeding in nursing rats (unpublished observations by Fleming, 1974, and J. M. Stern, 1974, cited in Wade, in press).

The present results also extend the findings of Stern and Zwick (1973) to prepubertal, pregnant, pseudopregnant, and lactating rats: NE infused intraventricularly during the dark decreased feeding and increased activity. Furthermore, while nearly

15% of the animals studied by Stern & Zwick became ill after infusion, only two animals showed signs of sickness in the present study. Most importantly, this work utilizes relatively more physiological dosages of NE; Stern and Zwick employed 50-250 micrograms NE. The level of NE found in the entire rat brain approximates 1 microgram (de la Torre, 1972).

Table 4 and the results of Stern and Zwick demonstrate that NE can suppress feeding in rats. These data are in contrast to the commonly held view that NE stimulates eating (Grossman, 1962; Slangen & Miller, 1969). Viewing NE as a feeding inhibitor gains support from the meticulous work of Margules and his group (Margules, 1970a; Margules 1970b; Margules, Lewis, Dragovich & Margules, 1972). One way out of this stimulator-suppressor dilemma is to assume that feeding is managed by dopamine and that NE modulates this basic neurochemical system. Zigmond and Strickler (1972; 1973), using 6-hydroxydopamine, have established the significance of dopamine and the nigrostriatal tract in the control of feeding (see also Marshall, Richardson, & Teitelbaum, 1974). Following the lead of Margules et al. (1972), it is suggested that noradrenergic neurons originating in the periventricular-medial hypothalamus modulate the lateral hypothalamic feeding system. Low and high levels of noradrenergic stimulation decrease activity in the feeding system while moderate levels increase activity. By adding exogenous NE to the already high levels of NE found in the rat brain during darkness (Manshardt & Wurtman, 1968), it is assumed that the present work produced high levels of NE stimulation and feeding inhibition. Again, this formulation allows one to reconcile the present findings with the mass of evidence demonstrating norepinephrine's ability to stimulate eating.

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(Received for publication August 1, 1975;
accepted August 11, 1975.)