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Effects of Ovariectomy and Estradiol Injections on Food Intake and Body Weight in Rats with Ventromedial Hypothalamic Lesions[1]

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BEATTY, W. W., D. A. O'BRIANT AND T. R. VILBERG. *Effects of ovariectomy and estradiol injections on food intake and body weight in rats with ventromedial hypothalamic lesions.* PHARMAC. BIOCHEM. BEHAV. 3(4) 539–544, 1975. – Female rats with lesions of the ventromedial hypothalamus (VMH) were ovariectomized during the static obese stage after body weight levels had stabilized. Following ovariectomy, rats with VMH lesions showed smaller increases in food intake and less body weight gain than non-lesioned controls ovariectomized at the same time. Subsequently, the effects of peripheral injections of estradiol benzoate (EB) on feeding and body weight were examined. Ovariectomized rats with VMH lesions were also less responsive to exogenous EB treatment; they lost significantly less weight in response to estrogen than controls. EB caused a somewhat smaller reduction in food intake by the VMH group but this difference was not significant. Considered together the available data on changes in responsiveness to endogenous and exogenous estrogen following VMH lesions suggest a role for VMH estrogen receptors in the regulation of body weight, but these estrogen receptors may not modulate weight by directly altering food intake as previously suggested.

Feeding Body weight regulation Estrogen Ventromedial hypothalamic lesions

OVARIAN hormones, especially estrogen, exert important regulatory influences upon food intake and body weight in females of nearly all mammalian species (see [8, 20, 22] for reviews). Following ovariectomy in the adult, food intake temporarily increases; body weight increases and is thereafter regulated at a chronically higher than normal level [20]. Estrogen, but not progesterone treatment can prevent or reverse these effects of ovariectomy [6, 8, 15, 19].

The work of Wade and Zucker [23], since replicated in two other laboratories [1,7], indicates that the ventromedial hypothalamus (VMH) may contain estrogen receptors which are important to the regulation of food intake. In the majority of cases, implants of crystalline estradiol benzoate (EB) into the VMH of ovariectomized females resulted in depressed food intake; at these same sites testosterone, progesterone or cholesterol did not typically affect feeding. Implants of EB in hypothalamic areas other than the VMH were usually ineffective as well [23]. If, as Wade and Zucker suggested, the VMH is the focal point for estrogenic modulation of feeding, then rats with VMH lesions should exhibit smaller increases in food intake and gain less weight than nonlesioned controls following ovariectomy. Conversely, they should also show smaller reductions in feeding and less weight loss following peripheral treatment with EB.

The published evidence germane to these predictions does not reveal the anticipated changes in food intake and body weight following experimental manipulations of estrogen in animals with VMH lesions. Valenstein and his coworkers [18] reported that ovariectomy performed 21 or 70 days prior to VMH lesions did not affect the magnitude of hyperphagia or body weight gain induced by the lesions. King and Cox [10] performed ovariectomies and VMH lesions at the same time and concluded that their effects on food intake and body weight were independent and additive. Moreover it has been reported that animals with VMH lesions are at least as sensitive as controls to the anorexic effects of peripheral EB [10] or diethylstilbestrol [12].

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However, careful inspection of the data presented by King and Cox [10] suggests that body weight changes following ovariectomy or EB injections were somewhat smaller in magnitude in the animals with VMH lesions. The present study was designed to reexamine the effects of ovariectomy and EB injections on food intake and body weight in animals with VMH lesions. Since both the magnitude and duration of hyperphagia are often quite variable among animals with comparable lesions, we sought to quantify the effects of estrogen in rats with VMH lesions more precisely by performing ovariectomies and EB treatments on animals whose body weights and food intakes had stabilized at the levels typical of the static obese stage.

METHOD

Animals

Adult female rats of the Holtzman strain were used. They were caged singly and housed in an air-conditioned animal room maintained on a 12:12 light–dark cycle. Purina lab chow pellets and water were freely available in the home cage.

Surgery and Histology

Operations were performed under Chloropent anesthesia (2.0 cc/kg; Fort Dodge Lab, Fort Dodge, Iowa) supplemented with 0.05 mg atropine sulfate. Bilateral lesions of the VMH were produced by passing 1.6 mA DC for 10 sec (LVE 1644 Lesion Maker) between a 0 ga stainless steel insect pin stereotaxically implanted into the brain and a rectal cathode. The insect pin, insulated with Epoxylite, was scraped to expose the tip. With the head held approximately horizontally between bregma and lambda the coordinates for the VMH lesions in mm with respect to bregma were: 2.7 posterior, 0.5–0.6 lateral, 8.6 below the surface of the cortex. In control animals the scalp was incised and the skull was bared, but the electrode was not lowered into the brain. At the conclusion of testing all rats with lesions were sacrificed with an overdose of Chloropent and perfused with physiological saline and 10% Formalin. The brains were frozen and sectioned at 40 μ on a cryostat. Every fifth section through the lesion was saved and stained with cresylviolet. Lesions were reconstructed with the aid of a microprojector with reference to the plates of the König and Klippel [11] atlas. A detailed quantitative analysis of lesion size and location was conducted [19], but inasmuch as correlations between these anatomical measures and the several behavioral indices were not statistically significant, no further description will be provided here.

Procedure

The experiment was performed in two replicates with slight differences in procedure. In the first replicate the rats were 5–7 months old at the time of brain surgery; all had previously served as untreated controls in various behavioral experiments. Prior test history was not confounded with surgical treatment. Body weight data were collected on 17 rats with VMH lesions and 14 control animals. Food intake was also measured for 12 animals in each condition. Following brain surgery, body weights were monitored daily until the rats gained less than 1 g/day over a one week period. Food intake and body weight were monitored for the next 7 days. The average values on these measures were taken as the preovariectomy baselines. All rats were ovariectomized and food intake and body weight were continously monitored from the third postoperative day until the end of the experiment. Ovarian weights were determined at the time of ovariectomy. On the 29th and 30th day after ovariectomy all rats were injected subcutaneously with sesame oil (0.20 cc/kg) which had no discernable effect on either food intake or body weight in either group. Since previous work [2] demonstrated that a 32-day series of sesame oil injections permitted substantial weight gain in ovariectomized females, a more sustained sequence of oil injections seemed superfluous. For the next 12 days all animals received subcutaneous injections of EB (Progynon benzoate in sesame oil, Schering; 4 μg/kg of a 20 μg/cc solution). To assess the effects of EB on food intake and body weight, the mean food intake on Days 22–30 was used as a baseline, while mean body weight on Days 29–30 served as the baseline for this measure. The effects of ovariectomy and EB treatments on both food intake and body weight were assessed by analyzing change scores computed by subtracting performance during each stage of the measurement period from the appropriate baseline.

In the second replicate, experimentally naive animals that were 90–110 days old were used. VMH lesions (N = 21) or control operations (N = 11) were performed as described above. When the animals reached the postoperative criterion for stability of body weight (less than 1 g/day weight gain over a two week period), preovariectomy baseline values were determined for body weight and food intake. Mean values for food intake and body weight over this 14 day period were used as baselines. Following ovariectomy, changes in food intake and body weight were studied for a nine week period. This procedural change was made because the data from the first replicate suggested that the static obese animals with VMH lesions might be recovering more slowly than controls from the surgical trauma associated with ovariectomy. Accordingly, the postovariectomy measurements were extended and more stringent pre-EB baseline values were established. Prior to initiating EB treatment all animals met a criterion of less than 1 g/day weight gain over a one week period. Body weight and food intake levels during this 7 day period served as baselines against which the EB treatment effects were assessed. After baseline values were established for food intake and body weight each animal in both groups received two days of oil injections followed by 30 days of EB injections using procedures identical to those of the first replicate. As in the first replicate, the effects of VMH lesions on the response to ovariectomy and EB treatment were assessed by analyzing difference in scores computed by subtracting the mean performance on each measure from the appropriate baseline.

RESULTS

Figures 1 and 2 depict changes in food intake and body weight consequent to ovariectomy. Despite differences in the length of the postovariectomy measurement period, rats with VMH lesions gained less weight than controls, $F(1,29) = 8.46$, $p<0.01$ for Replicate 1 and $F(1,30) = 6.01$, $p<0.025$ for Replicate 2. Of particular importance, there was no suggestion that differences in body weight between the VMH and control groups were becoming smaller during the second month of the postoperative recovery period for the second replicate. Although there was a slight suggestion

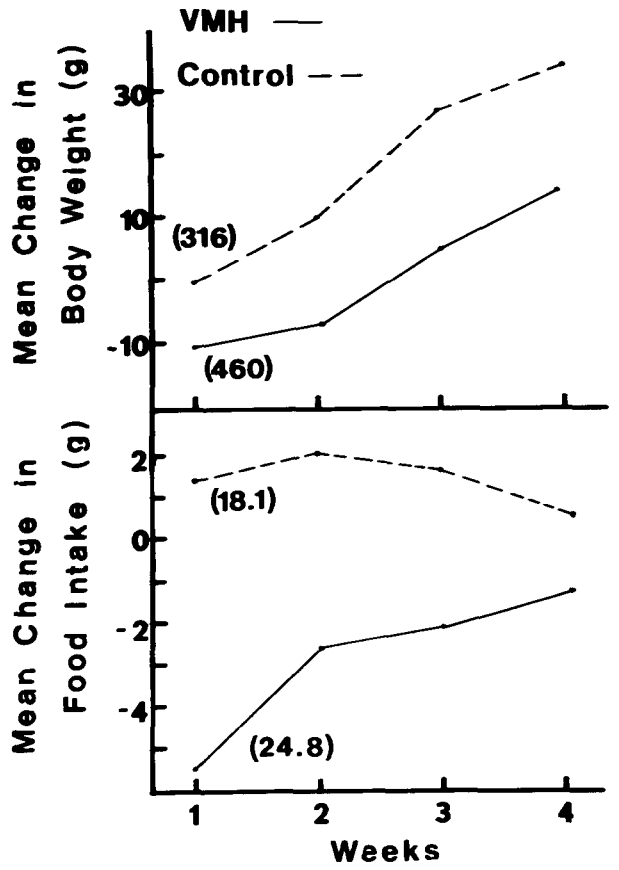

FIG. 1. Effects of ovariectomy on changes in body weight and food intake in rats with VMH lesions and in controls in Replicate 1. Numbers in parentheses are the preovariectomy baseline values from which the change scores were computed. For body weight the change scores were computed by subtracting the baseline from the weight on Days 7, 14, 21, and 28. For food intake the change scores were computed by subtracting the baseline from the mean consumption of Days 4–7, 8–14, 15–21, and 22–30.

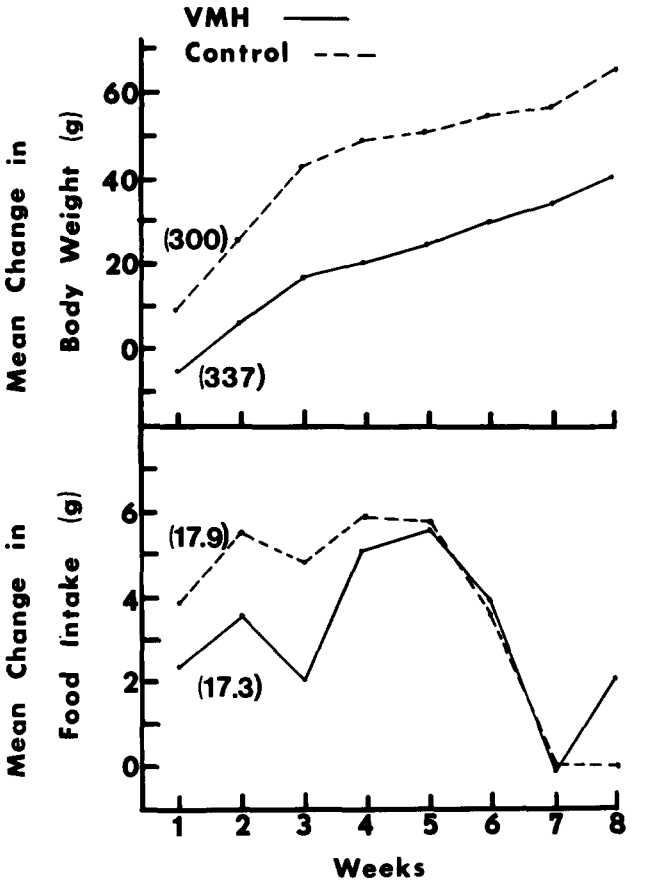

FIG. 2. Effects of ovariectomy on changes in body weight and food intake in rats with VMH lesions and in controls in Replicate 2. Numbers in parentheses are the preovariectomy baseline values from which the change scores were computed. For body weight the changes were computed by subtracting the baseline from the mean weight on Days 6–8, 13–15, 20–22, 27–29, 34–36, 41–43, 48–50, and 55–57. For food intake the change scores were computed by subtracting the baseline from the mean consumption on Days 5–9, 12–16, 19–23, 26–30, 33–37, 40–44, 47–51, and 54–58.

that the weight curves were converging for the first replicate, this trend was not at all distinct.

As might be expected, group differences in food intake tended to parallel the changes observed in body weight. In the first replicate, where the VMH group had a significantly higher baseline food intake level than controls ($U = 10.5$, $p<0.01$), controls exhibited a slight increase in food intake following ovariectomy while the rats in the VMH group actually exhibited an initial depression in food intake, especially during the first postoperative week. Overall, the group difference on the food intake change measure was highly significant, $F(1,22) = 21.05$, $p<0.001$. In the second replicate, where the VMH and control groups did not differ significantly in baseline food intake levels, both groups exhibited an initial increase in food intake following ovariectomy; the magnitude of change was greater for the control group. As a result, there was a significant Lesion X Weeks interaction, $F(7,210) = 4.33$, $p<0.001$, although the main effect of Lesion was not significant.

Changes in food intake and body weight during EB treatment are shown in Figs. 3 and 4. In terms of body weight change, the animals with VMH lesions were less responsive to the weight-suppressing effects of EB treatment in both replicates; $F(1,29) = 11.10$, $p<0.005$ for Replicate 1; $F(1,30) = 4.43$, $p<0.05$ for Replicate 2. The results of the second replicate are of particular importance, since the initial body weight levels for the VMH and control groups were quite comparable at the start of EB treatment.

Similar, but less clear cut effects were observed on the food intake measure. In both replicates animals with VMH lesions tended to exhibit smaller depressions in food intake than rats in the control group, but in neither case did the differences attain an acceptable level of significance; $F(1,22) = 3.06$, $p<0.10$ for Replicate 1 and $F< 1$ for Replicate 2. We also analyzed all of the data in terms of the percentage change from the appropriate baseline. All of the differences described above with absolute change scores were also observed on the percent change measures. In addition, for Replicate 1 animals with VMH lesions exhibited a signifi-

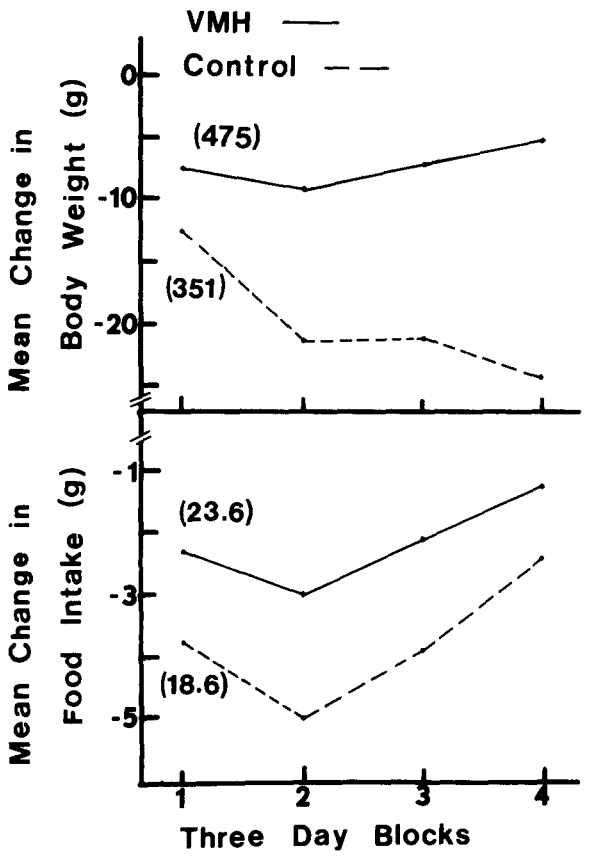

FIG. 3. Effects of EB treatment on changes in body weight and food intake in rats with VMH lesions and in controls in Replicate 1. Numbers in parentheses are the pretreatment baseline values from which the change scores were computed. For body weight the change scores were computed by subtracting the baseline from the weight on Days 3, 6, 9 and 12. For food intake change scores were computed by subtracting the baseline from the mean consumption on Days 1–3, 4–6, 7–9, and 10–12.

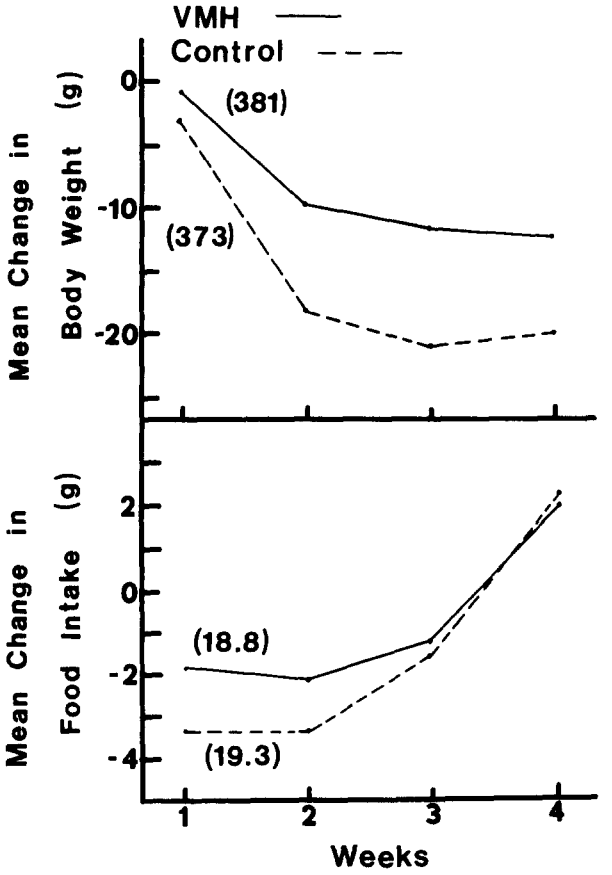

FIG. 4. Effects of EB treatment on changes in body weight and food intake in rats with VMH lesions and in controls in Replicate 2. Numbers in parentheses are the pretreatment baseline values from which the change scores were computed. For body weight the change scores were computed by subtracting the baseline from the mean weight on Days 6–8, 13–15, 20–22, and 27–29. For food intake the change scores were computed by subtracting the baseline from the mean consumption on Days 5–9, 12–16, 19–23, and 26–30.

cantly smaller reduction in food intake during EB treatment than controls.

Figure 5 depicts two representative lesions reconstructed on the plates of the König and Klippel [11] atlas. Rat OB 18 sustained damage typical of the smaller lesions in the series while Rat OB 31 is representative of the larger lesions. In both instances extensive bilateral damage to the VMH and arcuate nuclei was sustained. The larger lesions also damaged portions of the anterior hypothalamic nuclei and the dorsomedial nuclei as well as the region immediately lateral to the VMH nuclei. In general the lesions in the first replicate were somewhat larger than in the second. In addition, two animals in the second replicate sustained lesions which damaged only the most posterior portions of the VMH nuclei, but destroyed most of the ventral hypothalamus posterior to the VMH and anterior to the mammillary nuclei. We analyzed all the data with and without these two rats and the conclusions reported are unaffected by the inclusion of these subjects.

DISCUSSION

The present findings demonstrate that when compared to controls, static obese rats with VMH lesions gain less weight and show smaller increases in food intake following ovariectomy. The data also reveal that animals with VMH lesions lose less weight but show nearly normal suppression of food intake in response to peripheral EB injections. In a very general sense these observations are consistent with the hypothesis that the VMH area contains receptors which are important to body weight regulation, a view which is supported by findings indicating that implants of crystalline EB into the VMH but not elsewhere in the hypothalamus inhibits food intake and body weight [1, 7, 23]. However, the present findings appear to contradict other reports [10, 12, 18] that animals with VMH lesions retained at least normal responsiveness to the weight-limiting effects of estrogen.

One obvious procedural difference between the present and the earlier studies is that we assessed the effects of ovariectomy and EB injections on female rats with VMH lesions whose body weights had reached the static obese plateau. In other studies with female rats the ovariectomies

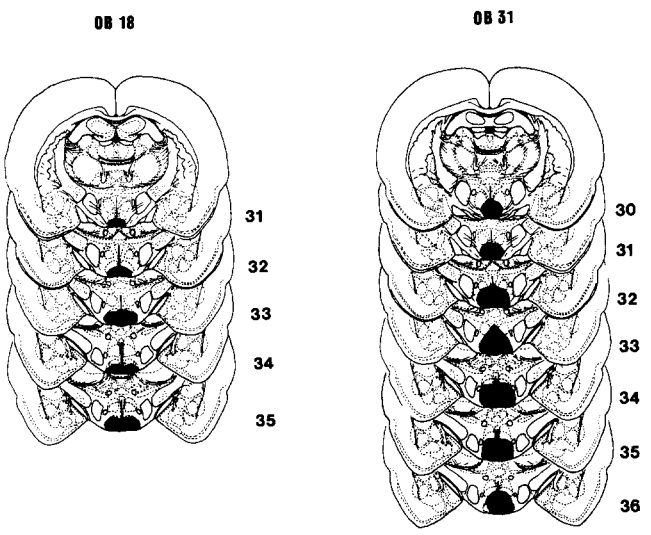

FIG. 5. Reconstructions of the largest (OB 31) and smallest lesions (OB 18) from the two replicates. Numbers to the right of the sections refer to plates in the atlas of König and Klippel [11].

were performed prior to [18] or at the same time [10] as the VMH lesions.

While our procedure has the advantage of permitting assessment of the effects of ovariectomy and EB treatment in the same animals, precluding the possibility that subtle differences in the location or size of the lesions could complicate the interpretation of the results, there remains the possibility that the weight level characteristic of the static obese stage represents a physiological ceiling. This possibility is supported by the fact that the magnitude of weight gain following ovariectomy in lesioned animals was inversely related to the magnitude of weight gain following production of VMH lesions but before ovariectomy ($r = -0.50$, $p<0.05$ for Replicate 1; $r = -0.54$, $p<0.025$ for Replicate 2). Obviously this inverse relationship could also be explained by assuming that lesions which caused greater weight gains prior to ovariectomy destroyed more of the estrogen receptors in the VMH area.

Alternatively, it could be argued that ovariectomy caused smaller increases in food intake and body weight in the lesioned animals because the VMH lesions reduced circulating levels of estrogen prior to ovariectomy. This view is supported by reports of gonadal atrophy following VMH lesions [3,4], although we did not observe significant changes in ovarian weight resulting from the VMH lesions produced in the present experiments.

Finally, differential recovery from the surgery of ovariectomy may have contributed to the difference in the response to ovariectomy between the VMH and control groups. In the first replicate there was some suggestion that the lesioned group was gaining weight more rapidly than the controls at the end of the ovariectomy period. Concern for this possibility led us to extend the postovariectomy period to 8 weeks in the second replicate. Although there was no suggestion that the growth curves were converging by the end of the 8 week period, the fact that the growth functions for the VMH and control groups were essentially parallel over the entire 8 week period could be explained by assuming that the animals in the VMH group were initially more responsive to the surgical trauma associated with ovariectomy and thereafter gained weight in a normal fashion.

Any of the above explanations can account for the effects of ovariectomy on food intake and body weight in animals with VMH lesions. However, the present finding that EB injections resulted in reduced weight loss by the lesioned animals cannot be explained by the assumption that the static obese weight level is a physiological ceiling, nor can it be accounted for by assuming that VMH lesions reduced endogenous estrogen. Even if the VMH and control groups did respond differentially to the surgical trauma of ovariectomy, it is not clear how this fact could account for the reduced effectiveness of exogenous EB in suppressing body weight among the lesioned animals unless one assumes that the EB treatment was initiated when the controls had reached a stable postovariectomy weight level but while the lesioned animals were continuing to gain weight. This amounts to assuming that the postovariectomy growth curves of the two groups would have ultimately converged, had the measurements been more extended. Examination of the data and procedure of the second replicate suggests that this possibility is rather remote for two reasons: (1) EB injections did not begin until all of the animals exhibited minimal weight gains following ovariectomy and (2) by the seventh week after ovariectomy food intake had returned to the preovariectomy baseline for both the VMH and control groups. In short, the hypothesis that the VMH area (which may also include the arcuate nuclei) contains estrogen receptors which are important to body weight regulation most adequately explains the reduced responsiveness to ovariectomy and EB treatment observed in the present study. That EB injections reduced weight loss in the lesioned rats is all the more striking in light of several recent reports that the weight-limiting effects of estrogen are usually greater in heavier animals [13, 17, 25]. Moreover, several recent and as yet unpublished reports indicate that under certain circumstances rats with VMH lesions may be less than normally responsive to the suppressive effects of estrogen on food intake or body weight [5, 14, 22].

The data reported by Goldman and Bernardis [5] are of particular interest in light of the present findings that EB injections caused normal suppression of food intake but less than normal reduction of body weight in animals with VMH lesions. Goldman and Bernardis observed that estrogen increased weight gain in weanling male rats with VMH lesions, but reduced weight gain in controls. In their study all animals received the same amount of food. In fact, an examination of the data presented by King and Cox [10] suggests a similar dissociation between changes in food intake and body weight in animals with VMH lesions in response to experimental manipulation of estrogen levels. In the King and Cox study the magnitude of weight gain induced by ovariectomy appears to be less in animals with VMH lesions than in controls; furthermore, it also appears that the suppressing effect of exogenous EB on body weight gain was less in females with VMH lesions than in controls. In the same animals changes in food intake following ovariectomy or estrogen treatment were as large or larger in the lesioned rats as in controls.

Taken together the available data suggest that the VMH contains receptors which monitor estrogen and affect body weight regulation. While there is ample evidence that neurons in the VMH can directly inhibit feeding when exposed

to estrogen [1, 7, 23] they apparently only do so if body weight exceeds a certain level [7]. In the absence of VMH receptors other brain or peripheral loci evidently can monitor estrogen and reduce feeding normally, although suppression of body weight is not as great as when the VMH receptors are intact. This dissociation between changes in food intake and body weight in response to estrogen treatment in animals with VMH lesions may occur because estrogen does not produce the usual increase in activity in the lesioned animals [9] or it may result from complex changes in endocrinemetabolic function consequent to the VMH lesions (see e.g. [5, 16, 21, 24]).

Since peripheral EB treatment revealed a dissociation between changes in food intake and body weight in rats with VMH lesions, it is surprising that a similar dissociation was not observed in response to ovariectomy. This may suggest that other ovarian hormones exert more important effects on the regulation of food intake or body weight when the VMH is destroyed or it may indicate that peripheral EB injections in the dose employed in the present study do not completely mimic the actions of endogenous estrogens.

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