Lesions of hypothalamic paraventricular nuclei do not prevent the effect of estradiol on energy and fat balance

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Dagnault, Anne, and Denis Richard. Lesions of hypothalamic paraventricular nuclei do not prevent the effect of estradiol on energy and fat balance. *Am. J. Physiol.* 267 (*Endocrinol. Metab.* 30): E32–E38, 1994.—The chronic effects of estradiol (E_2) on energy balance have been investigated in ovariectomized rats with hypothalamic paraventricular nuclei (PVH) lesions. Body weight and food intake were monitored throughout the E_2 treatment, which lasted 26 days. At the end of this treatment, rats were decapitated, and their carcasses were processed to determine the body contents in energy, fat, and protein. Plasma adrenocorticotropic hormone (ACTH) and corticosterone were determined by radioimmunoassay and protein-binding assay at the end of the study. Regardless of whether they were sham- or PVH-lesioned, E_2-treated rats ate, expended, and gained significantly less energy than untreated animals. In addition, E_2-treated rats deposited less fat and protein than the rats not receiving E_2. In contrast to the E_2 treatment, PVH lesions accelerated the gains in energy and fat regardless of whether the rats were treated with E_2 or with a placebo. There were no interaction effects of PVH lesions and the E_2 treatment on energy or fat gains. Plasma levels of corticosterone and ACTH were higher in E_2-treated rats than in animals receiving the placebo treatment. The present results provide evidence that the hypothalamic PVH is not an essential neuroanatomical structure in the effects of E_2 on energy and fat balances.

paraventricular nucleus of hypothalamus; ovariectomy; body composition; body weight

IT HAS BEEN KNOWN FOR YEARS that the experimental treatment with estrogens impairs the gain or causes the loss of body weight in rats (27,37). In addition, blunted weight gains have been observed during the estrus cycle, after the preovulatory surge of estradiol (E_2; see Ref. 37). This loss or reduced gain in body weight induced by estrogens reflects a negative energy balance; estrogens lead to an anorectic effect that is not outweighed by a concomitant reduction in energy expenditure. Estrogens have not been convincingly shown to stimulate brown adipose tissue thermogenesis (27) but may be capable of promoting energy expenditure via increasing muscular activity (37).

The mechanism and site of action of estrogens in the regulation of energy balance have only been scantily addressed. Lately, by preventing the anorectic effects of E_2 with the antagonist α-helical corticotropin-releasing factor (CRF), Dagnault et al. (10) provided evidence for the involvement of CRF in the anorectic effects of E_2. CRF, whose first recognized action relates to the control of the pituitary-adrenal axis (1), is a neuropeptide widely distributed in the brain (32) that also exerts numerous behavioral (15) and autonomic functions (4), including anorectic (3, 12) and thermogenic (28, 31) effects. Indirect support for the stimulating effects of estrogen on CRF neuronal activity has emerged from numerous experiments which emphasized the activating role of estrogen on the pituitary-adrenal secretions (5). Recently, the suggestion was made that the site for the anorectic action of estrogen could be the hypothalamic paraventricular nucleus (PVH; see Refs. 6, 7). The PVH contains numerous neuroactive substances liable of affecting regulation of energy balance that include CRF, oxytocin, cholecystokinin, dynorphin, vasopressin, galanin, and neurotensin (19, 39). Because PVH has the highest hypothalamic concentration of CRF-containing cells (32), it has to be considered as a possible site for the CRF-mediated effects of estrogens on energy balance. The present study was carried out with the specific aim of assessing the chronic effects of E_2 on energy balance of ovariectomized female rats with PVH lesions.

MATERIALS AND METHODS

Animals and diet. Female Wistar rats with an initial body weight of 240–260 g were purchased from The Canadian Breeding Laboratories (St. Constant, Canada). All rats were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, which is approved by the Natural Sciences and Engineering Research Council of Canada. The animals were housed singly in wire-bottomed cages suspended above absorbent paper. They were maintained under controlled temperature (23 ± 1°C), on a 12:12-h light-dark cycle, with the lights turned off between 1800 and 0600 h. Each animal had free access to water and to a purified diet, the energy density of which was 17.62 kJ/g wet wt. The diet contained the following components (in g/100 g): 23.0 casein, 0.3 methionine, 38.6 dextrose monohydrate, 24.5 corn starch, 2.5 cellulose, 5.0 corn oil, 5.0 mineral mix (AIN 76), and 1.0 vitamin mix (Teklab test diets, no. 40060).

Procedures. A few days after delivery to the animal room, the rats were surgically ovariectomized, PVH lesioned, and then divided into four groups. Each of the four groups received one of the following treatments: *1*) sham lesions with placebo treatment, *2*) PVH lesions with placebo treatment, *3*) sham lesions with E_2 treatment, and *4*) PVH lesions with E_2 treatment. The bilateral ovariectomies and the electrolytic PVH lesions were achieved after an intraperitoneal injection of 3 ml/kg of an anesthetic mixture consisting of ketamine (20 mg/ml) and xylazine (2.5 mg/ml). With the incisor bar placed at 3.75 below the interaural line, a tungsten microelectrode (A-M Systems, Everett, WA) left uninsulated over a 0.05-cm tip was positioned according to the following coordinates from the atlas of Paxinos and Watson (26): 1.6 mm caudal to bregma, 0.4 mm lateral to the midline, and 7.9 mm ventral from the surface of the skull. A 0.75-mA direct anodal current was delivered during 30 s. In the sham-lesion group, the electrode was lowered to 1.0 mm above the PVH; no current was passed. E_2 (Sigma Chemicals, St. Louis, MO) was administrated through 6-mm-long implants that were subcutaneously implanted in the middorsal region (29). The implants consisted of Silastic tubing (3.2 mm OD, 1.5 mm ID) packed with

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crystalline E_2 over a length of 5 mm and plugged at both ends with silicone adhesive. Silastic implants have been used repeatedly to achieve constant release and to maintain uniform circulating levels of estrogens (17, 18, 34). Empty implants were used as placebos. The implants were installed while the rats were ovariectomized and PVH lesioned.

Energy balance measurements. The body weight was monitored every 2nd day throughout the period of treatments, which lasted 26 days. Similarly, the amount of food ingested, which was corrected for the spillage, was also measured and cumulated. At the end of the treatment, rats were killed by decapitation at 1100 h. Gastrointestinal contents were removed from each rat carcass before being autoclaved at 125 kPa for 20 min to soften hard tissues. This procedure has not been reported to affect energy yield (23). The autoclaved carcass was homogenized in a volume of distilled water equal to 1.5 times its weight. A sample of the homogenized carcass was then freeze-dried, pending the determination of the contents in energy and nitrogen. The energy content of the carcasses as well as that of the diet were determined by adiabatic bomb calorimetry (Parr Instruments, Moline, IL) calibrated with a dry benzoic acid standard. Carcass nitrogen was determined in 250- to 300-mg samples of dehydrated carcass using the Kjeldahl procedure. The carcass content in protein was computed by multiplying the carcass content in nitrogen by 6.25. The energy as protein was subtracted from total body energy of the carcass to determine the energy as nonprotein matter. Because carbohydrate represents a negligible part of total carcass energy (38) the energy from nonprotein matter was assumed to be essentially that of fat. Such an assumption tends to be confirmed by studies in which energy, fat, and protein were directly determined (2). Values of 23.5 and 39.3 kJ/g were taken (38) for the energy content of protein and fat, respectively. The initial carcass contents in energy, fat, and protein of the rat were estimated from the body weights by reference to the baseline group killed at the beginning of the experimental period. Such estimations allow the determination of the gains in energy, fat, and protein for the treatment period. The 10 rats in the baseline group were killed at the beginning of the energy balance trial, and their carcasses were individually analyzed for fat, protein, and energy. Then, the body weight densities in fat (g fat/g body wt), protein (g protein/g body wt), and energy (kJ energy/g body wt) were computed and averaged. The average densities were then multiplied by the initial body weight of each rat ascribed to the experimental groups. Rats in the initial group were, in every aspect (strain, age, gender), identical to the four experimental groups.

Hormone assays. At the time of decapitation, blood was collected, and plasma was frozen at −70°C. Plasma corticosterone was determined by a competitive protein-binding assay (25) using the plasma of a female rhesus monkey as the source of transcortin. The plasma levels of adrenocorticotropic hormone (ACTH) were measured by immunoassay (Allegro ACTH immunoassay kit, Nichols Institute, San Juan Capistrano, CA). This ACTH immunoassay incorporates a monoclonal antibody and a polyclonal antibody, both with high affinity and specificity for defined amino acid regions of the ACTH molecule. The precision (or intra-assay variance) and the reproducibility (interassay variance) are 3.0 and 6.8%, respectively.

Histological analysis. At the time of death, the brains were removed and fixed in a solution of formaldehyde (10%) containing sucrose (10%). Frozen sections were cut (40-μm slices) and stained with thionine (NISSL stain). Each lesion was carefully visualized under a microscope, and only rats with ≥80% bilateral damage to the parvocellular division of the PVH were retained and included in subsequent analyses (Fig. 1). The parvocellular division of the PVH comprises most of the

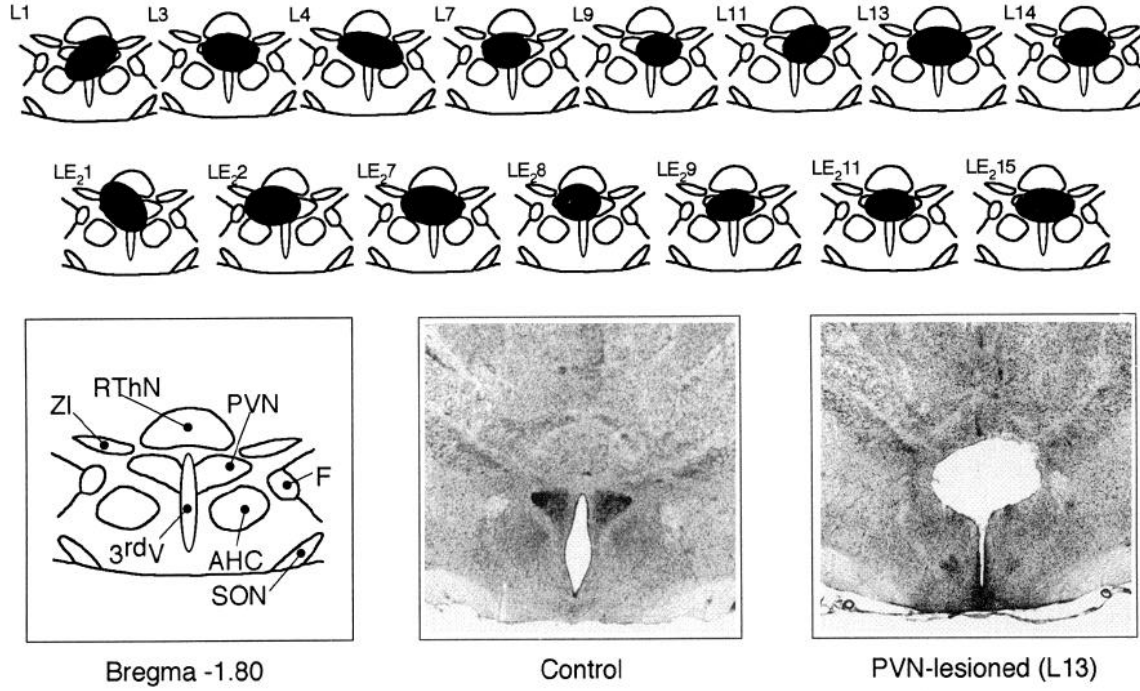

Fig. 1. Histological localization of hypothalamic paraventricular nucleus lesions (PVH). *Top*: drawings illustrating extent of PVH damage in rats retained in energy balance measurements. Drawings were achieved from 40-mm thick slice of brain anteroposteriorally cut at bregma −1.8 (see *bottom left*; Ref. 26). *Bottom middle* and *right*: brain slices of control and lesioned animals, respectively. For more details, see MATERIALS AND METHODS. L, lesioned; E_2, estradiol; 3^{rd}V, third ventricle; AHC, anterior hypothalamus central; F, fornix; RThN, reuniens thalamic nucleus; SON, supraoptic nucleus; ZI, zona incerta.

CRF-containing cells perikarya within the PVH. Eight out of 15 rats were retained in the lesioned group treated with the placebo, whereas 7 out of 15 rats were retained in the lesioned group treated with E_2. The drawings of the lesions of the rats that were considered for energy balance data analyses can be seen on Fig. 1. It is worth pointing out that the histological analyses were realized not only on the slice (~bregma −1.8) that was used for the drawings in Fig. 1 but also on slices covering a range of slices exceeding PVH anteriorly and posteriorly, that is, from bregma −0.92 to bregma −2.5.

Statistics. The data were analyzed using a 2 × 2 factorial analysis of variance (ANOVA) to determine the main and interaction effects of the factors "PVH lesion" and "E_2 treatment," each having two levels (PVH lesion: "sham lesions" and PVH lesion; E_2 treatment: "placebo" and E_2 treatment). When an interaction occurred, the statistical significances of the difference between individual means were tested a posteriori with the Dunn-Sidak procedure.

RESULTS

Body weight. Figure 2 expresses the body weight growth curves and the body weight gains of the various groups used in this study. ANOVA revealed a significant PVH lesion-E_2 treatment interaction on the total body weight gain; the PVH lesion led to an increase in the total body weight gain in E_2-treated animals but not in rats receiving a placebo. As evident from the graph and confirmed by a posteriori comparisons, PVH lesions were incompetent in preventing the effect of E_2 on the body weight gain.

Food intake. The effects of the PVH lesion and the E_2 treatment on the cumulative and cumulated intakes are shown in Fig. 3. The cumulated intakes represent the amounts of food ingested either during the first half of the study or during the whole experimental period. During the first half of the study, the result analysis showed the opposite effects of the PVH lesion and the E_2 treatment on food intake. There was no interaction effect of the PVH lesion and the E_2 treatment on food intake. From *day 2* to *day 12*, PVH-lesioned rats ate significantly ($P < 0.05$) more food than sham-lesioned animals, regardless of whether they were treated with E_2. Similarly, E_2-treated animals ate less food than animals with empty implants regardless of wether they were PVH lesioned or intact. When the whole cumulated intake is considered, the data analysis revealed a significant "PVH lesions-E_2" interaction. As shown in Fig. 3 and confirmed by a posteriori comparisons the significant interaction was accounted for by the fact that PVH lesions did not affect food intake in placebo animals, whereas it led to a significant increase in ovariectomized animals. As also confirmed by a posteriori comparisons, PVH lesions proved inefficient in preventing the effect of E_2 on total cumulated food intake.

Energy balance and body composition. Energy intake, energy expenditure, and energy gain were significantly ($P < 0.05$) higher in PVH-lesioned rats than in sham-lesioned animals and, in contrast, significantly ($P < 0.05$) lower in E_2-administered rats than in placebo-implanted animals (Table 1). Figure 4 illustrates the respective and opposite effects of the PVH lesion and the treatment with E_2 on the gains in body protein and fat. PVH-lesioned groups exhibited a significantly ($P < 0.05$) higher fat gain than the sham-lesioned groups, whereas E_2-treated animals deposited significantly ($P < 0.05$) less protein and fat than the placebo-treated animals.

Because rats with only 80% of the PVH parvocellular division lesioned were included in the data analyses, one might relate the inability of PVH lesions to prevent the effects of E_2 on energy balance to the fact that the PVH nuclei were not damaged enough. To assess this possibility, independent analyses were carried out on the animals with damage that extended to the whole parvocellular division of the PVH structure. These analyses produced results comparable to those emerging from the analyses that included all of the data. In fact, in using the lesioned rats (L3, L4, L13, L14, LE_27, LE_29, LE_211,

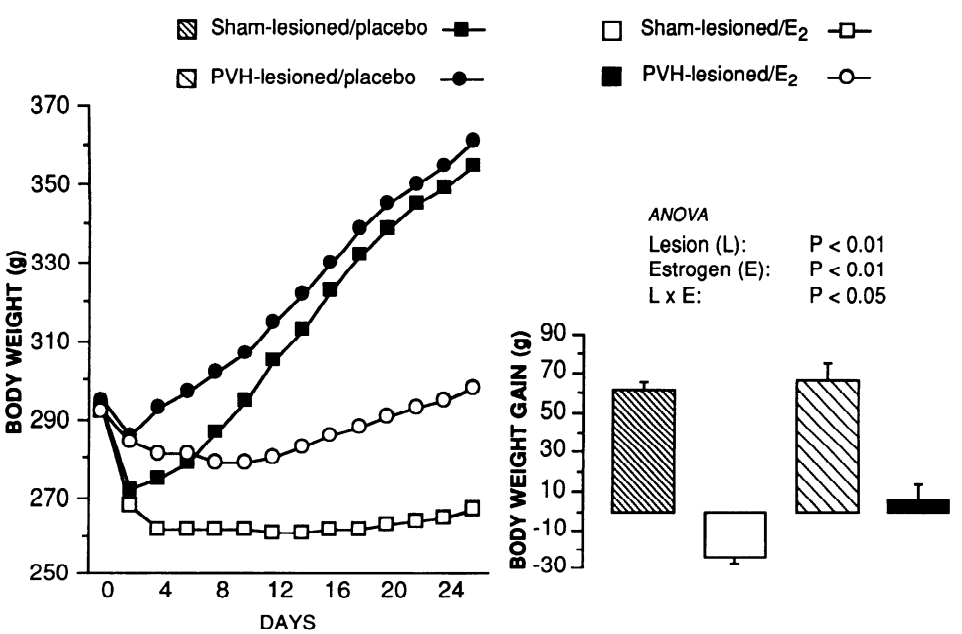

Fig. 2. Body weight growth curves and body weight gains (bar graph) of various groups used in this study. Means ± SE are expressed. No. of rats were 9 in each sham-lesioned group and 7 and 8 in PVH-lesioned groups treated with E_2 and placebo. For more details on statistics, see RESULTS.

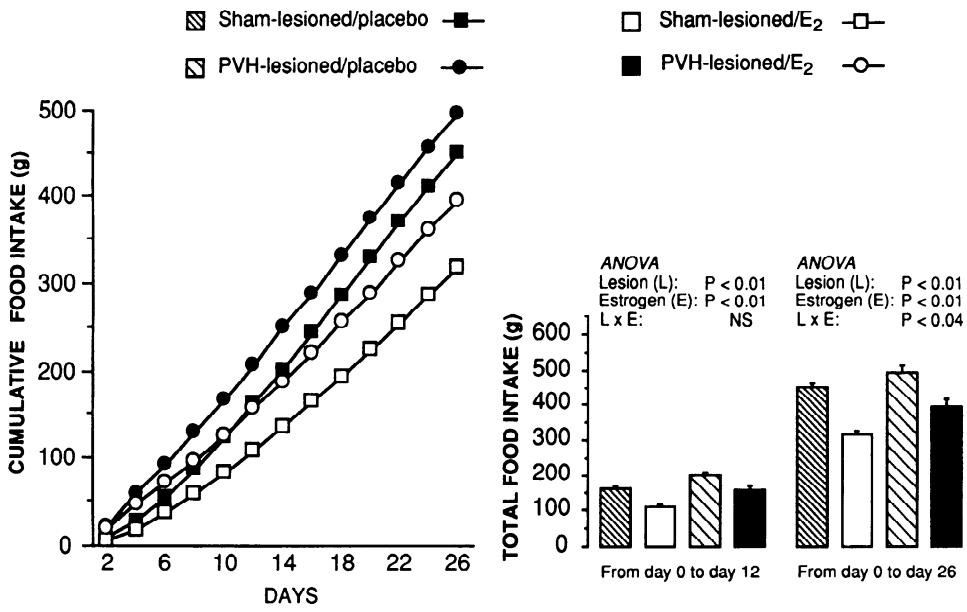

Fig. 3. Cumulative and total (bar graphs) food intakes of various groups used in this study. Means ± SE are expressed. No. of rats were 9 in each sham-lesioned group and 7 and 8 in PVH-lesioned groups treated with E_2 and placebo. For more details on statistics, see RESULTS.

$LE_2$15), the mean energy gains were 704 ± 121, −699 ± 114, 1,234 ± 337, and −279 ± 441 for, respectively, the sham-lesioned/placebo, the PVH-lesioned/placebo, the sham-lesioned/E_2, and the PVH-lesioned/E_2 groups, and ANOVA revealed significant main effects of PVH lesion (P = 0.0385) and E_2 treatment (P = 0.0001) without significant PVH lesion-E_2 treatment interaction (P = 0.8013).

Plasma ACTH and corticosterone levels. Figure 5 expresses the plasma levels of ACTH and corticosterone after 4 wk of treatment. E_2 treatment induced a significant ($P < 0.05$) increase in plasma levels of corticosterone and ACTH in both PVH-lesioned and sham-lesioned groups. PVH lesions had no significant effect on plasma levels of corticosterone and ACTH.

Table 1. *Energy intake, expenditure, and balance in PVH-lesioned and E_2-treated animals*

	Energy, kJ		
	Digestible intake	Gain	Apparent expenditure
Sham lesioned			
Placebo	7,554 ± 204	704 ± 121	6,850 ± 252
Estradiol	5,312 ± 155	−699 ± 114	6,011 ± 146
PVH lesioned			
Placebo	8,316 ± 308	1,299 ± 165	7,018 ± 174
Estradiol	6,621 ± 364	−29 ± 292	6,651 ± 202
ANOVA			
L	0.0002	0.001	0.0384
E	0.0001	0.0001	0.0065
L × E	0.0411	0.8300	0.2347

Values represent means ± SE; n = 9 rats in each of the sham-lesioned groups and 7–8 rats in hypothalamic paraventricular nucleus (PVH)-lesioned groups treated with estradiol (E_2) and placebo. Digestible energy intake was estimated as 95.5% of gross energy intake by reference to previous experiments carried out in ovariectomized rats (9). For more details on statistics, see RESULTS. ANOVA, analysis of variance; L, lesion; E, estrogen.

DISCUSSION

The present study provides evidence that the PVH does not represent the essential brain site of estrogen action on energy and, perhaps more evidently, on fat balance. Indeed, the present results indicate that a bilateral lesion of the PVH, capable, per se, of promoting energy and fat gain is not sufficient to prevent the influence of E_2 on energy balance of female rats. The chronic E_2 treatment led to reduction in energy gain regardless of whether this treatment was administered to sham- or PVH-lesioned rats.

The inability of the PVH lesion approach to alter the CRF-mediated effects of estrogen on energy balance emerges from this study as an unexpected finding. Indeed, there are sound reasons to suspect that PVH, which contains estrogen receptors (33) and the highest hypothalamic concentration of CRF-containing cell bodies (32), may be essential as a neuroanatomical site in the effects of estrogens in the regulation of energy balance. In a recent investigation carried out in rats, PVH implants of diluted E_2 have been shown to specifically reduce food intake without altering the reproduction-related lordosis behavior (6). It is worth pointing out that PVH, notwithstanding its potential implication in the effects of estrogens on energy balance, is recognized as a dominant structure in the regulation of energy balance. This role of PVH has been emphasized in studies implicating the PVH as a significant and specific site of action of various neurotransmitters and neuropeptides in the control of energy intake. PVH has been, for instance, implicated as the specific site of the orexigenic actions of both norepinephrine (21) and neuropeptide Y (21, 35) or the anorectic effects of both serotonin (22) and CRF (16). Sound evidence for a role of PVH in the regulation of energy balance has also come forth from the studies demonstrating the obesity-inducing effects of the PVH lesion approach (13, 14, 20). The present results confirm that the PVH lesion acceler-

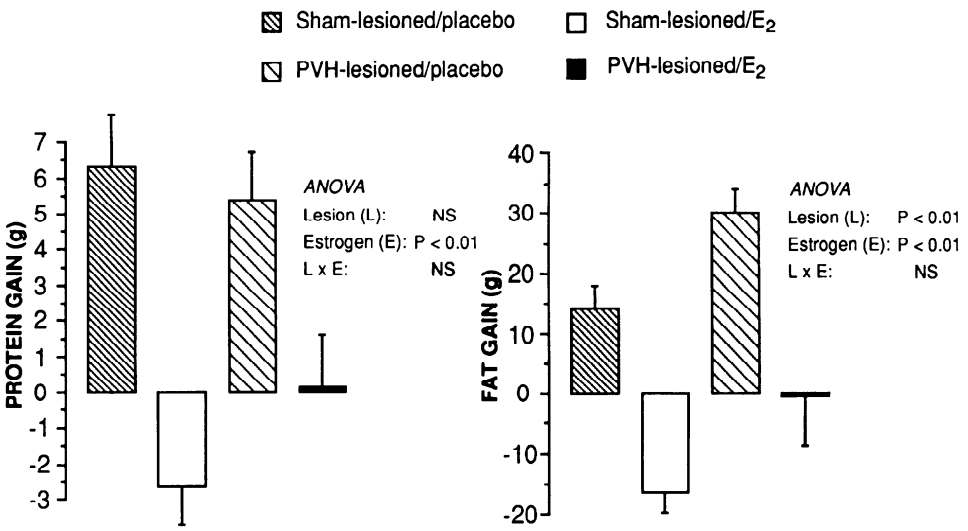

Fig. 4. Protein and fat gains of various groups used in this study. Means ± SE are expressed. No. of rats were 9 in each sham-lesioned group and 7 and 8 in PVH-lesioned groups treated with E_2 and placebo. For more details on statistics, see RESULTS.

ates body fat and energy deposition. Leibowitz et al. (20) reported a gender effect of the PVH lesion on body weight, with the obesity-inducing effect of the lesion being stronger in females than in males. The present results, which show that PVH lesions can promote energy deposition in OVX rats, indicate that the possible gender influence of the PVH lesions on energy balance is not solely due to ovarian hormones. It is also noteworthy that PVH lesions do not invariably cause obesity in rats (11), and it is has been suggested that large lesions that may include extra PVH areas such as the perifornical structures may be more efficient at inducing obesity than small lesions (11, 13, 36).

Although the inability of PVH lesions to prevent the CRF-mediated effects of estrogens on fat and energy balance represents a somewhat surprising observation, it is nonetheless conceivable. In fact, it is thoroughly possible that some CRF estrogen-sensitive neurons involved in the regulation of energy balance can be located outside the PVH. Incidentally, the present results do not invariably rule out the involvement of PVH in the estrogen action in the regulation of energy balance of intact animals. PVH lesions may lead to compensatory adjustments that prevent the estrogen effects from occurring. It is worth pointing out that the PVH lesion approach has repeatedly been proven to be deficient at preventing the effects of various treatments on energy balance-related variables, even when circumstances give strong support for the lesion approach. PVH lesions, for instance, have been proven to be inefficient in preventing the anorectic effects of exercise (30), choleckystokinin, and LiCl (11) as well as the orexigenic effects of glucoprivic or lipoprivic substances such as 2-deoxyglucose and mercaptoacetate (8). Interestingly, the effect of PVH lesions on the pituitary adrenal secretion can also be compensated even if PVH contains the CRF cell bodies of the CRF neurons responsible for triggering the pituitary secretion of ACTH. Such a compensation has been reported before by Makara et al. (24), who have shown that the effects of PVH lesions do not blunt the pituitary-adrenal response to stress after 6 wk of treatment. In agreement with this finding, there was no effect of PVH lesions on the levels of plasma ACTH or corticosterone in the present study.

Even if the present results do not support a role for the PVH in the long-term effects of estrogens on energy and fat balance, they do not totally exclude the possibility that the PVH may be implicated in the effect of

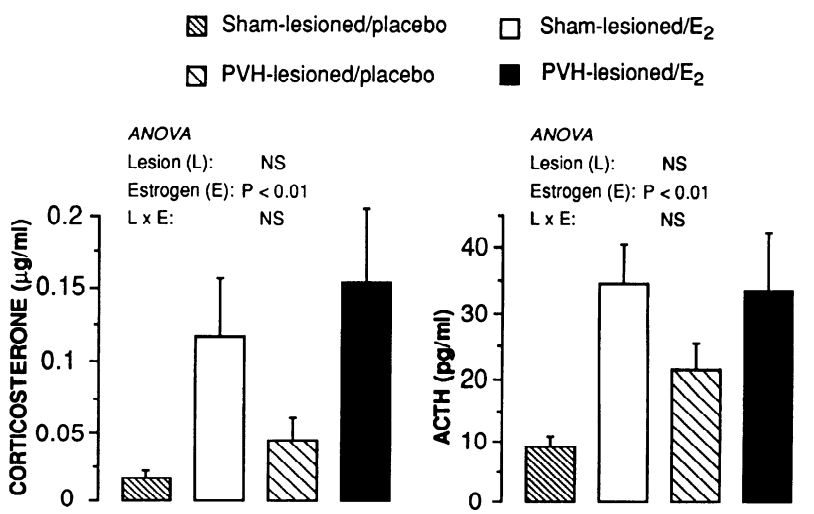

Fig. 5. ACTH and corticosterone of various groups used in this study. Means ± SE are expressed. No. of rats were 9 in each sham-lesioned group and 7 and 8 in PVH-lesioned groups treated with E_2 and placebo. For more details on statistics, see RESULTS.

estrogens on protein balance. ANOVA revealed a close to significant PVH lesion-E_2 treatment interaction ($P = 0.1$) on the protein gain (Fig. 3). The effects of E_2 in reducing the protein gain tended to be less marked in the PVH-lesioned animals than in nonlesioned animals. This effect of PVH lesions in the effect of E_2 on the protein gain may confer an explanation for the observation that the effect of E_2 on body weight was less pronounced in PVH-lesioned rats. The observation that PVH lesions can partially block the effect of E_2 on the protein gain is plausible. In fact, E_2 may stimulate the secretion of corticosterone, which exerts a potent catabolic effect on the protein mass. Considering the key involvement of PVH in the secretion of the glucocorticoids, it may be reasonable to suggest that the PVH may mediate the effects of E_2 on the glucocorticoid secretion and therefore on the protein catabolism. It is consequently logical to consider that the lesion of the PVH may prevent the effects of E_2 on protein gain. However, if the effect of PVH lesions in preventing the effect of estrogens on the protein gain is mediated by the effect the lesion is exerting on the glucocorticoid secretion, a reduction of the corticosterone secretion should have been observed in PVH-lesioned animals treated with E_2. However, no reduction was observed, as revealed by the results of Fig. 4. Because corticosterone levels were determined only at the end of the study, the results do not rule out the possibility that PVH lesions may have reduced, for a few days after the lesion, the effects of E_2 on the secretion of corticosterone and hence on the protein and the weight gain. The weight gain results expressed on Fig. 2 seem to be in agreement with this suggestion.

Recently, Butera et al. (7) suggested that the effects of E_2 on food intake may require the integrity of the PVH. Butera et al. (7) indicated that PVH lesions could prevent the anorectic effect of E_2. Intriguingly, Butera et al. (7) did not prevent, by damaging the PVH, the lowering effect of E_2 on body weight and water intake. Although the study of Butera et al. (7) may indicate a role for the PVH in the acute anorectic effect of E_2, they did not include any energy balance and body composition measurements and therefore, in terms of the effects of E_2 in the regulation of energy balance, should be interpreted with caution. The observed effect might represent an acute effect, which could vanish relatively rapidly; the results of the present study clearly demonstrate that PVH lesions could not prevent the effect of E_2 in the long term.

In conclusion, the present study provides evidence that the PVH does not represent an essential brain site of estrogen action in the regulation of energy balance. Essentially, the present results indicate that the bilateral lesion of the PVH is not sufficient to prevent the influence of neither the administration nor the removal of E_2 in the regulation of energy balance of female rats. E_2 chronic treatment and ovariectomy, respectively, led to a reduction and increase in fat and energy gain regardless of whether these experimental treatments were administered to sham- or to PVH-lesioned rats.

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