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Research Article

Effects of a Brain-Enhanced Chemical Delivery System for Estradiol on Body Weight and Food Intake in Intact and Ovariectomized Rats

James W. Simpkins,[1,3,4] Wesley R. Anderson,[1,3] Ralph Dawson, Jr.,[1] and Nicholas Bodor[2,3]

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Studies were undertaken to determine the effects on body weight of a brain-enhanced chemical delivery system for estradiol. This estradiol-chemical delivery system (E_2-CDS) has a long half-life in the brain, where it slowly releases estradiol but is quickly cleared from peripheral tissues. We administered, by a single iv injection, E_2-CDS (0.2, 1.0, or 5.0 mg/kg), equimolar doses of another 17-hydroxy-substituted estrogen, estradiol valerate (E_2-VAL), or the dimethyl sulfoxide (DMSO) vehicle to female rats. Daily food intake and body weight was determined for 24 days thereafter. E_2-CDS caused an initial dose-dependent suppression in body weight for up to 8 days and a suppression in food intake for up to 4 days. In response to E_2-VAL, the initial declines in body weight and food intake were lower in magnitude, were shorter in duration, and showed no dose dependency. Following this period of weight loss, E_2-CDS-treated rats gained weight at a rate greater than that of the DMSO controls, and at the 0.2- and 1.0-mg/kg doses, body weights achieved were greater than control levels. To determine the role of the ovaries on this biphasic response to E_2-CDS, long-term ovariectomized rats were treated with E_2-CDS (1.0 mg/kg) or the vehicle and parameters of body weight regulation were determined for 25 days. Ovariectomized rats responded to E_2-CDS with a prompt and sustained decrease in body weight which did not recover over the 25-day course of the study. The body-weight loss in ovariectomized rats was associated with a marked reduction in food intake for 8 days. Finally, when intact female rats were administered the E_2-CDS on the day of diestrus I, rats exhibited cornified vaginal epithelial lavages for 3.5 days, during which weight loss was observed, followed by a 7.8-day period of pseudopregnancy during which animals rapidly gained weight. Collectively, these data indicate that delivery of E_2 to the brain with E_2-CDS causes a marked decline in body weight and food intake in female rats. The phase of increased body weight which follows this drug-induced weight loss appears to be ovarian dependent, since in ovariectomized rats this phase of response to the drug is not observed.

KEY WORDS: estradiol; estradiol delivery system; food intake; body weight.

INTRODUCTION

Estradiol (E_2) is a physiological modulator of body weight and food intake behavior in a variety of mammals including the rat (1,2), guinea pig (3), ewe (4), pigtailed monkey (5), baboon (6), rhesus monkey (7–9), and human female (10,11). Elevated E_2 during the follicular phase of the ovarian cycle is associated with reduced food intake and weight loss, while elevated progesterone during the luteal phase of the ovarian cycle is associated with enhanced food intake and body-weight gain (1–6,8–13). Ovariectomy results in a marked increase in body weight in rats and this effect is blunted by replacement of E_2 (1,2,14–16) but not progesterone (1,2). E_2 delivered locally to the periventricular or ventromedial nucleus of the hypothalamus (17–19) exerts effects on body weight, food intake, and lipoprotein lipase activity which are similar to those seen following systemic administration of the gonadal steroid, suggesting a central locus of action for the weight-reducing effects of E_2. Additionally, localized lesions of hypothalamic and septal regions can reduce or blunt the inhibitory effects of E_2 on body-weight gain in ovariectomized rats (1,2).

That E_2 can modulate food intake and body weight in human subjects is suggested by the observation that women consume up to 40% fewer calories during the follicular phase of the menstrual cycle than during the luteal phase (10). However, despite this evidence for a modulatory role of E_2 in body-weight regulation in a variety of species, the potential for the therapeutic use of estrogens to reduce body

[1] Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida 32610.
[2] Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610.
[3] Center for Drug Design and Delivery, College of Pharmacy, University of Florida, Gainesville, Florida 32610.
[4] To whom correspondence should be addressed at Box J-487, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610.

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weight in obese patients has not been evaluated for two reasons. First, estrogens have a wide distribution in the body (20) and the presence in many tissues of estrogen receptors creates the potential of untoward peripheral side-effects. Second, treatment with oral contraceptives which contain both estrogen and progestagens has inconsistent effects on body weight (20). The potential therapeutic use of estrogens to achieve weight reduction has not been evaluated.

We have developed a chemical delivery system for the brain-enhanced delivery of drugs. This chemical delivery system is based upon the *in vitro* covalent binding of a lipophilic dihydropyridine moiety to the drug and the *in vivo* oxidation of the dihydropyridine to a pyridinium ion (21). The lipophilic dihydropyridine allows drugs readily to cross the blood–brain barrier and *in situ* oxidation to the pyridinium ion slows the egress of the drug from the central nervous system (21). While the lipophilic estradiol can readily penetrate the blood–brain barrier, it can also redistribute back to the periphery as blood levels of the steroid decline. In contrast, the formation in the brain of the charged quaternary salt of the delivery system slows the redistribution of the delivery system and subsequent hydrolysis of the pyridinium ion results in the slow release in the brain of estradiol (22–24).

Because of the potential usefulness of this estradiol-chemical delivery system (E_2-CDS) as a probe for separating centrally mediated from peripherally mediated effects of estradiol, we undertook an evaluation of the effects of E_2-CDS on body weight and food intake in female rats.

MATERIALS AND METHODS

Drug Synthesis and Administration

The synthesis of the E_2-CDS has been described by us in detail (22–24). Briefly, the 2,17β-dinicotinate ester of E_2 was made by refluxing 17β-E_2 with nicotinoyl chloride or nicotinic anhydride in hyridine. This derivative was selectively hydrolyzed to the 17-monoester of E_2 with potassium bicarbonate in 95% methanol. The monoester of E_2 was then quaternized with methyl iodine. The delivery system was then prepared by reduction of the monoester of E_2 with $Na_2S_2O_4$. The structure of each intermediate and final product was confirmed by nuclear magnetic resonance and elemental analysis: mp 115–130°C dec; NMR ($CDCl_3$) δ 7.0–6.8 (m, 2H, C-1 E_2 proton + C-2 pyridine H), 5.0–4.5 (m, 3H, C-17δ E_2 + C-5 pyridine + phenolic OH, exchangeable), 3.2–3.0 (m, 2H, C-4 pyridine protons), 3.0–2.9 (s, 3H, NCH_3), 2.9–1.1 (m, 15H, E_2 skeletal H's), 1.0–0.9 (s, 3H, C-18 E_2 protons). The yields at each synthetic step were 64–94%.

Estradiol valerate (E_2-VAL) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Both drugs were dissolved in dimethyl sulfoxide (DMSO). E_2-CDS was administered at doses of 0.2, 1.0, or 5.0 mg/kg body wt in a volume of 0.5 ml DMSO/kg. E_2-VAL was diluted in DMSO to achieve doses which were equimolar to that of E_2-CDS. DMSO-treated rats served as vehicle controls in each study. Intravenous injection (tail vein) was the preferred route of administration because the pharmacokinetics of the E_2-CDS are well described with this and no other routes of administration (22–24). E_2-VAL was chosen as a positive control for comparison with E_2-CDS since it, like E_2-CDS, is substituted at the 17 position and, as a result, shows decreased metabolism and an enhanced half-life (20). This ensured that any differences in the duration of the response were not due simply to the metabolic protection provided by substitution at the 17 position of estradiol.

Animals

Female Charles River CD rats were purchased from the Wilmington, Mass., colony and were individually housed in a temperature (26°C)- and light (lights on 0500 to 1900 hr daily)-controlled room and were provided food pellets (Purina Rat Chow 5001) and tap water *ad libitum* for at least 1 week prior to the initiation of their acclimation to the conditions for monitoring daily food intake.

Measurement of Food Intake and Body Weight

Food intake was determined by presenting each rat with 50–55 g of Purina Lab Chow (5001; Ralston Purina Co., St. Louis, Mo.) pellets in a glass petri dish at 0800 to 1000 hr. Twenty-four hours later, the remaining food was dried and weighed. Food spillage was recovered from the catch paper under the cage of each animal, then dried, and its weight was added to the final food value. There were no significant differences among groups or over time within groups in the amount of food spillage. At the beginning of each 24-hr period, fresh food was presented to the rats. On days during which food intake was not determined, uneaten food was removed and 50–55 g of fresh food was presented to the rats. Animals were acclimated to this feeding procedure for 4 days. Thereafter, baseline daily food intakes were determined for an additional 7 consecutive days. The initial food intake levels reported were the mean of the last 3 days of baseline recordings. On each morning, body weights of rats were determined using a Mettler animal balance (model P3N).

Experiment 1

Young adult female rats were administered a single iv injection of 0.2, 1.0, or 5.0 mg E_2-CDS/kg body wt or equimolar doses of E_2-VAL. The DMSO vehicle served as the control for both groups. Body weights and 24-hr food intake were determined daily for 15 days, then at 19 and 22 days after drug administration. All seven groups of rats were processed in the same animal room over the same time course. In this experiment, estrous cycles were not monitored prior to or after drug administration.

Experiment 2

Young adult female rats were monitored for the regularity of their estrous cycles by obtaining daily vaginal lavages. After at least two 4-day estrous cycles were observed, rats were treated with E_2-CDS (1 mg/kg, iv) or vehicle on the day of diestrus I. Vaginal lavages were obtained for the next 16 days and body weights were determined at days 0, 4, 7, 11 to 12, and 14 after treatment.

Experiment 3

Ovariectomized female rats were administered by a single iv injection, 1.0 mg E_2-CDS/kg body wt or the DMSO vehicle. Body weights and 24-hr food intake were determined daily for 15 days, then at 19 and 25 days after drug injection. Both groups were processed in the same animal room over the same time course.

Statistical Evaluation

Initial values for food intake and body weight were subjected to analysis of variance (ANOVA) and Student–Newman–Keuls (SNK) tests and no differences among group means were observed. All data were then normalized to the initial value for each animal (defined as 0) to reduce variability among animals within a treatment group. Data for food intake and body weight were then subjected to ANOVA and SNK tests for evaluation of the significance of effects of drug dose between treatment groups and for the effects of time after drug administration within treatment groups. For all tests a probability level of <0.05 was considered significant.

RESULTS

Experiment 1

The initial body weight for animals used in the E_2-CDS evaluation was 275 ± 4 g (mean ± SE) and means of individual groups did not differ significantly. Control rats treated with DMSO gained weight by 3 days postinjection and gained about 1 g body weight per day over the 22-day course of the experiment (Fig. 1). E_2-CDS caused a dose-dependent delay in the time until a significant increase in body weight was observed. Body-weight increases were seen with the 0.2-, 1.0-, and 5.0-mg/kg doses of E_2-CDS at 8, 9, and 11 days, respectively (Fig. 1). Relative to time 0, only the 5.0-mg/kg dose of E_2-CDS reduced body weight significantly from day 1 to day 5. Relative to DMSO controls, body weights were reduced from day 1 to day 3 at the 0.2-mg/kg dose, from day 2 to day 4 at the 1.0-mg/kg dose, and from day 1 to day 7 at the 5.0-mg/kg dose. After the initial decline, body weights exceeded those of DMSO controls for both the 0.2- and the 1.0-mg/kg dose groups (Fig. 1). From day 7 to day 14, the body weights of the 0.2-mg E_2-CDS/kg groups were significantly greater than those of controls, while at the 1.0-mg/kg dose, body weights exceeded controls from day 9 to day 14. From day 15 to day 22, body weights in all four groups were equivalent.

Initial 24-hr food intake levels were 17.4 ± 0.4 g and initial means of individual dose groups did not differ significantly. During the 22-day course of the study, food intake of DMSO controls differed from time 0 values on 2 days only (Fig. 2). The 0.2-mg/kg dose of E_2-CDS caused no significant changes in food intake relative to time 0 values or to DMSO control levels. However, both the 1.0- and the 5.0-mg/kg doses of E_2-CDS significantly, but transiently, reduced the 24-hr food intake (Fig. 2). Relative to time 0 values, the 1.0-mg/kg dose reduced the food intake from day 1 to day 2, while the 5.0-mg/kg dose reduced the food intake from day 1 to day 4. Late in the observation period, both of these dosage groups showed elevations in 24-hr food intake relative to

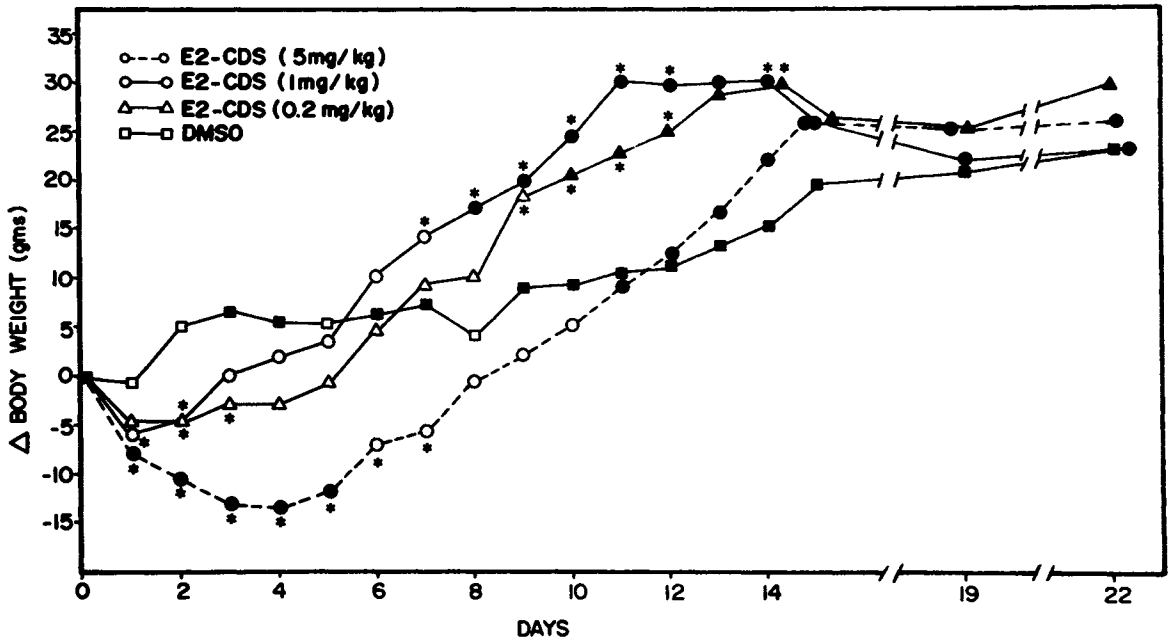

Fig. 1. Effects of E_2-CDS on body weight in female rats. Animals received a single intravenous (tail vein) injection of the vehicle (DMSO; □——□) or E_2-CDS at a dose of 0.2 mg/kg (○——○), 1.0 mg/kg (△——△), or 5.0 mg/kg (○- - -○). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values, and asterisks significant differences ($P < 0.05$) from the DMSO group at the same sampling time. Initial (Day 0) body weights did not differ significantly among groups and data for each rat were normalized to its initial body weight. The significance of differences among means was determined by ANOVA and SNK tests. $N = 7$ rats per group. The animals represented here were processed in the same room and on the same days as those for E_2-VAL in Fig. 3.

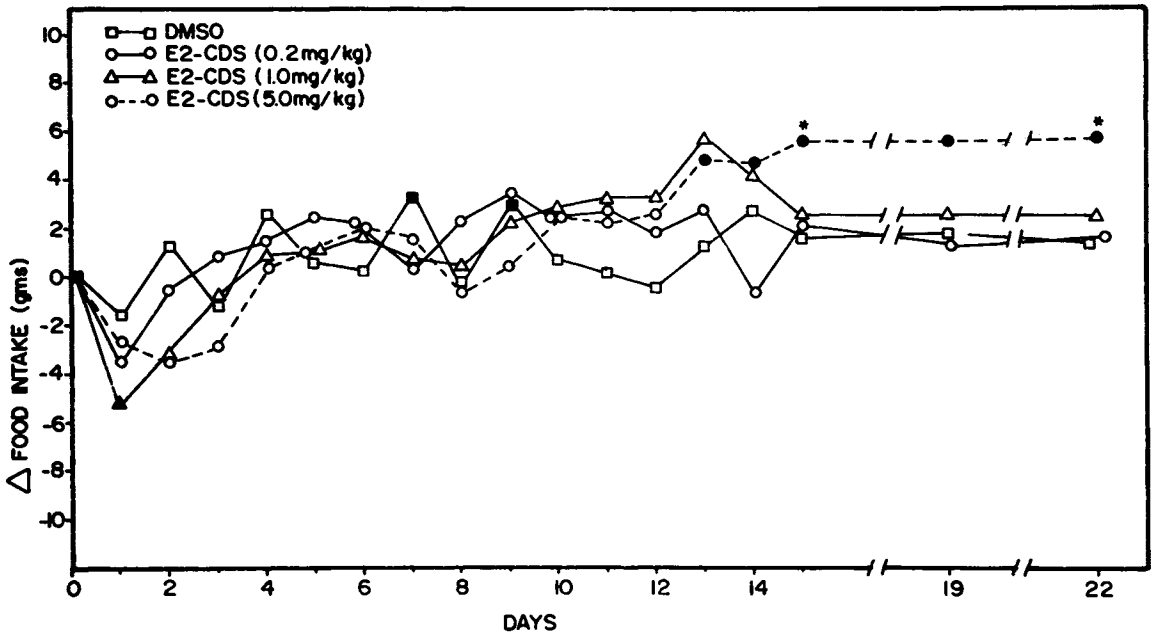

Fig. 2. Effects of E_2-CDS on daily food intake in female rats. Data are from animals depicted in Fig. 1. Symbols represent the response of animals treated with a single iv injection of vehicle (DMSO; □——□) or E_2-CDS at doses of 0.2 mg/kg (○——○), 1.0 mg/kg (△——△), or 5.0 mg/kg (○- - -○). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values, and asterisks indicate significant differences ($P < 0.05$) from the DMSO group at the same sampling time. Initial (Day 0) food intake values did not differ among groups and data from each rat were normalized to its initial level. The significance of differences among means was determined by ANOVA and SNK tests. $N = 7$ rats per group. The animals represented here were processed in the same room and on the same days as those for E_2-VAL in Fig. 4.

both time 0 and DMSO control levels. For the 1.0-mg/kg group, significant elevations in food intake occurred on days 8 and 13, while for the 5.0-mg/kg group, elevated food intake was observed from day 13 to day 22.

Initial body weights for animals used in the E_2-VAL study were 272 ± 3 g and initial mean values among dose groups did not differ. E_2-VAL, when administered in doses equimolar to that of E_2-CDS, caused a delay in the time until significant weight gain to 9, 10, and 11 days at the 0.2-, 1.0-, and 5.0-mg/kg doses, respectively (Fig. 3). Except for a reduction in body weight relative to DMSO controls on day 2, which showed no dose dependency, E_2-VAL caused little weight reduction. Similarly, no weight increase relative to DMSO controls was noted late in the observation period (Fig. 3).

Initial 24-hr food intake values for animals used in the E_2-VAL study were 16.8 ± 0.4 g and initial mean values did not differ among dosage groups. E_2-VAL caused a reduction in food intake only in the 1.0-mg/kg dose group during the first day after administration (Fig. 4). The two higher doses of E_2-VAL reduced food intake through day 4 when compared to DMSO controls. From day 13 to day 22, the 5.0-mg/kg dose of E_2-VAL resulted in elevations in food intake relative to time 0 values (Fig. 4).

Experiment 2

All 12 rats used in this study showed the expected 4-day estrous cycles (Fig. 5). Administration of E_2-CDS on diestrus I resulted in the appearance of a vaginal lavage predominated by cornified epithelial cells (i.e., an estrus-like lavage) which persisted for 3.5 ± 0.3 days (Fig. 5). In contrast, rats treated with the vehicle exhibited a leukocyte-predominated lavage the following day (the expected diestrus II pattern) and showed regular estrous cycles thereafter (Fig. 5).

In E_2-CDS treated rats, the period of drug-induced estrus was followed by 7.8 ± 1.1 days of leukocyte-predominated lavages indicative of the establishment of a pseudopregnant state (Fig. 5). By the 16th day after drug treatment, all but one rat had ovulated as evident by the appearance of epithelial cells in the vaginal lavage.

As observed in Experiment 1, treatment with 1 mg E_2-CDS/kg resulted in a significant reduction in body weight (−7.8 ± 2.3 g) by day 4 postinjection (Table I). Over the next 7–8 days, rats treated with the E_2-CDS gained weight rapidly, and from 11 to 14 days posttreatment their mean body weight exceeded that of vehicle-injected rats.

Experiment 3

Initial body weights in long-term ovariectomized (OVX) rats were 426 ± 12 g and initial body weights for the two treatment groups did not differ significantly. Over the 25-day time course of this study, body weights of DMSO-treated animals declined modestly from day 4 to day 14, then increased again late in the observation period (Fig. 6). In contrast, the 1.0-mg/kg dose of E_2-CDS caused a prompt (day 2) reduction in body weight which differed significantly both from time 0 and from DMSO controls from day 2 to day 25.

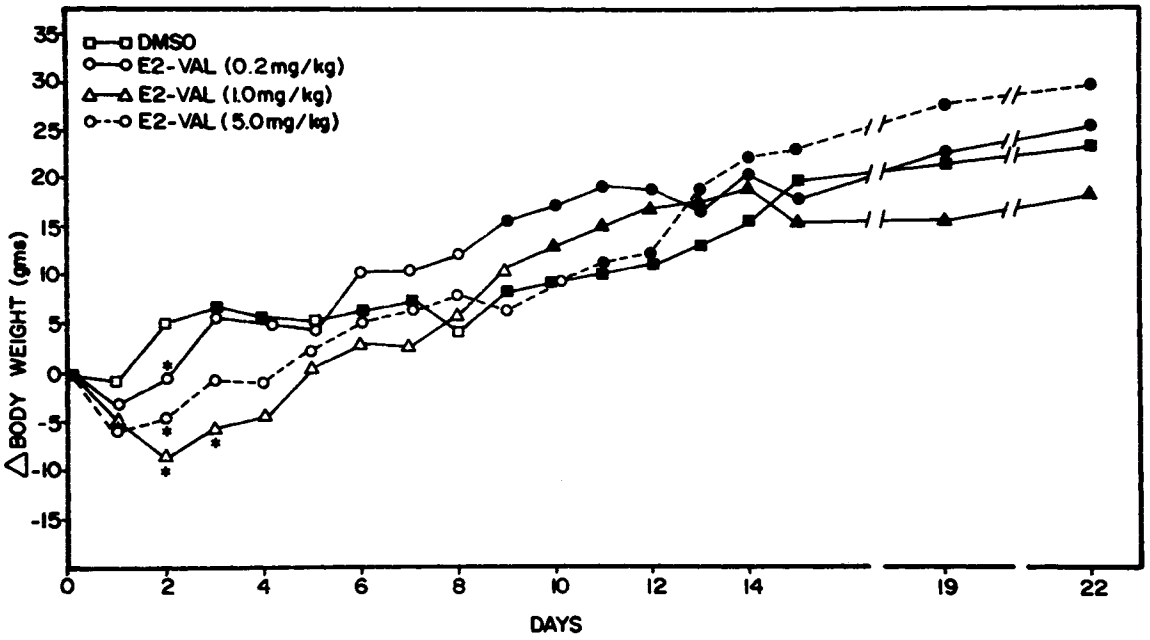

Fig. 3. Effects of E_2-VAL on body weights in female rats. Animals received a single intravenous (tail vein) injection of the vehicle (DMSO; □——□) or E_2-VAL at a dose of 0.2 mg/kg (○——○), 1.0 mg/kg (△——△), or 5.0 mg/kg (○- - -○). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values and asterisks indicate significant differences ($P < 0.05$) from the DMSO group at the same sampling time. Initial (Day 0) body weights did not differ significantly among groups and data for each rat were normalized to its initial body weight. The significance of differences among means was determined by ANOVA and SNK test. $N = 7$ rats per group.

By the last observation period we observed no evidence of significant recovery of body weight in the E_2-CDS-treated rats (Fig. 6).

Initial 24-hr food intake values for these OVX rats were 17.0 ± 0.6 g and initial food intake levels did not differ significantly between the two treatment groups. Daily food intake in DMSO-treated rats was stable except on days 5, 7, and 8, when an unexplained, significant reduction was noted (Fig. 7). In contrast, E_2-CDS caused a prompt (day 1) reduction in food intake which persisted through day 8 (Fig. 7).

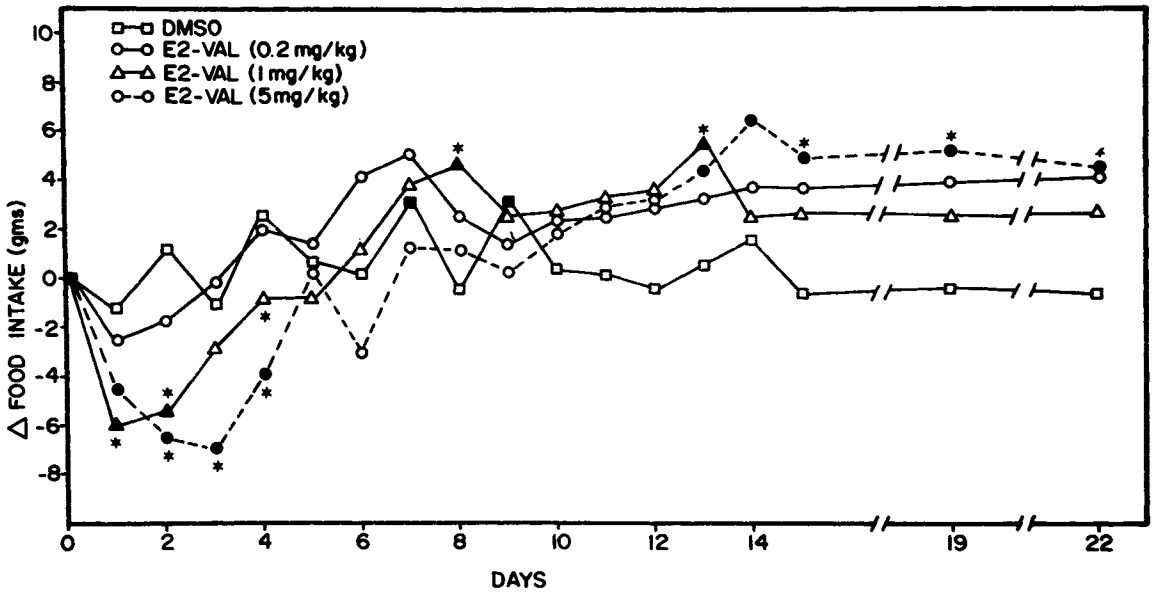

Fig. 4. Effects of E_2-VAL on daily food intake in female rats. Data are from animals depicted in Fig. 3. Symbols represent data from animals treated with vehicle (DMSO; □——□) or E_2-VAL at a dose of 0.2 mg/kg (○——○), 1.0 mg/kg (△——△), or 5.0 mg/kg (○- - -○). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values and asterisks indicate significant differences from the DMSO group at the same sampling time. Initial (Day 0) food intake values did not differ among groups and data from each rat were normalized to its initial level. The significance of differences among means was determined by ANOVA and SNK tests. $N = 7$ rats per group.

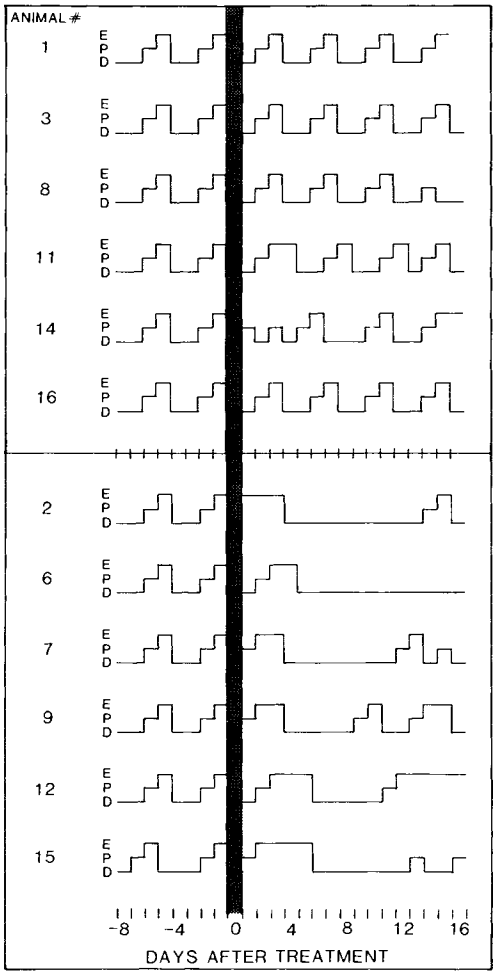

Fig. 5. Effects of E_2-CDS on the estrous-cycle pattern. The stippled area represents diestrus I, the day of treatment with vehicle (upper panel) or E_2-CDS (lower panel). The number on the left indicates the number assigned to the animal. E, estrus; P, proestrus; D, diestrus.

Thereafter, the food intake in the two groups was equivalent (Fig. 7).

DISCUSSION

This study reveals that E_2-CDS causes a biphasic effect on body weight in ovary-intact female rats but causes a prompt and sustained reduction in body weight in OVX animals. This biphasic effect of E_2-CDS was evident whether rats were administered the drug on diestrus I of the estrous cycle or randomly irrespectively of the estrous cycle day. Both the initial body-weight suppression and the subsequent body-weight increase were greater in E_2-CDS-treated rats than in animals treated with an equimolar dose of another 17-substituted estrogen, E_2-VAL. These data indicate that the brain-enhanced delivery of estradiol with this E_2-CDS has marked effects on body weight in the rat which appear to be modified by the presence of the ovaries.

The time course of body-weight alterations after a single iv dose of E_2-CDS is comparable to the duration of luteinizing hormone suppression in male and female rats (23,24) and the stimulation of masculine sexual behavior in male rats (25) following E_2-CDS treatment. The temporal coincidence of these two brain-mediated processes with the time course of weight change in female rats suggests that brain E_2 activity persists presumably from the chronic release of E_2 in the brain from the $E_2 - Q^+$. Estrogens, substituted in the 17 position, do not effectively bind to estradiol receptors and hence themselves have little estrogenic activity (26). This indicates that neither the delivery system itself nor $E_2 - Q^+$ formed in the brain is likely to account for the chronic effects of this drug. Rather, local brain E_2 release from the "locked-in" $E_2 - Q^+$ form of the delivery system is a more reasonable explanation of the persistence of the response.

This hypothesis is consistent with the pharmacokinetic behavior of E_2-CDS in rats. Following iv administration of E_2-CDS, the delivery system itself is cleared from the brain with a half-life of 29 min (22–24). In contrast, $E_2 - Q^+$ has a half-life in the brain of 24 hr (22) but is cleared from the liver, lung, and kidney with a half-life of 0.8, 5.5, and 7 hr, respectively (22). A subsequent study demonstrated that the half-life of $E_2 - Q^+$ in brain tissue is 6 to 10 times that of $E_2 - Q^+$ in kidney, heart, lungs, testes, and fat tissue (27). This long residency time of $E_2 - Q^+$ in brain, but not peripheral tissue, supports the proposal that E_2, generated locally from its hydrolytic cleavage from the charged pyridinium complex, is responsible for the long biological half-life of E_2-CDS. Since the duration of effect of E_2-VAL in this and our previous evaluations (23–25) was much shorter compared to E_2-CDS, reduced metabolism of E_2 caused by a 17-hydroxyl substitution of the steroid cannot account for the observed long biological effect of the E_2-CDS.

Changes in food intake after E_2-CDS treatment can explain part, but not all, of the resulting alterations in body weight in female rats. At the low dose of E_2-CDS (0.2 mg/kg), the initial body-weight reduction and subsequent body-weight increase were observed without a significant alteration in the daily food intake. At the other two doses of E_2-CDS, the phase of body-weight loss was associated with a marked reduction in the daily food intake. However, the role of food intake changes in the latter phase of body-weight gain is less clear since at the 1-mg/kg dose, no consistent hyperphagia was noted but rats achieved body weights greater than those of DMSO-control rats. Furthermore, rats treated with the 5-mg/kg dose of E_2-CDS showed a delayed hyperphagia (days 13 to 22) but did not exhibit body weights in excess of control animals. Hence, reduced food intake appears to be a component of the initial weight loss after E_2-CDS treatment in female rats, but changes in food intake are not a major component of the excess weight gain observed during the second week after E_2-CDS treatment.

Ovariectomy dramatically altered the response to E_2-CDS both qualitatively and quantitatively. In OVX rats, E_2-CDS caused a prompt and sustained weight reduction through the 25-day course of this study and no recovery of body weight was observed. Additionally, food intake was reduced through 8 days postinjection, a time period associated with the phase of rapid weight loss, and the normalization of food intake was associated with the maintenance of body weight at a level 30 to 40 g below preinjection levels. We observed no evidence of hyperphagia in OVX rats after E_2-CDS treatment.

Two differences between ovary-intact and OVX rats

Table I. Changes in Body Weight of Female Rats Administered E_2-CDS on Diestrus I of the Estrous Cycle

	Days after treatment				
	0	4	7	11–12	14
Vehicle	0	6.7 ± 3.3	8 ± 2.6*	20 ± 4.4*	22 ± 3.7*
E_2-CDS	0[a]	−7.8 ± 2.3*,**	2 ± 4.7	28 ± 4.1*	29 ± 4.7*

[a] Body weights of vehicle- and E_2-CDS-treated rats were 252 ± 5 (mean ± SE) and 252 ± 2 g, respectively.
* $P < 0.05$ vs day 0.
** $P < 0.05$ vs vehicle group at the same time.

could be involved in the differences in response to E_2-CDS noted in this study. First, the greater magnitude of decline in body weight noted in OVX rats may be due to the absence of circulating, endogenous estradiol and the resulting hyperresponsiveness of estrogen-responsive tissues. An increase in the amount of cytosolic receptors for estradiol is a well-described response to OVX (28). In the present study with E_2-CDS and in other studies which have utilized repeated doses of E_2 (1,2,16,29), OVX rats show a greater response to estrogens than ovary-intact animals. Thus initially, OVX rats are more sensitive to E_2-CDS than their counterpart cycling rats which have an endogenous source of estradiol.

Additionally, the blunting of the initial weight loss and the observation of subsequent weight gain in ovary-intact rats may reflect the ovarian progesterone response to E_2-CDS. E_2-CDS has been shown to suspend estrous cycles and elevate serum prolactin levels (30; Simpkins, unpublished observations). In the present study, we have observed that the E_2-CDS causes a persistent estrous state for about 4 days, during which weight loss is observed. Thereafter, rats exhibit a pseudopregnant condition which persists for the next 8 days and is associated with a phase of rapid weight gain. It is likely that E_2-CDS, through elevations in serum prolactin levels, causes rescue of the corpus luteum (31) and an elevation in serum progesterone associated with the resulting pseudopregnancy (32). As such, the elevation in progesterone would blunt the weight-reducing effects of E_2-CDS, as has been observed when progesterone is administered daily with estradiol (1,2,11,33,34). Later, the hormonal status of pseudopregnancy (i.e., elevated serum progesterone and declining brain E_2 levels) would be stimulatory to body-weight gain (16). In the absence of ovarian progester-

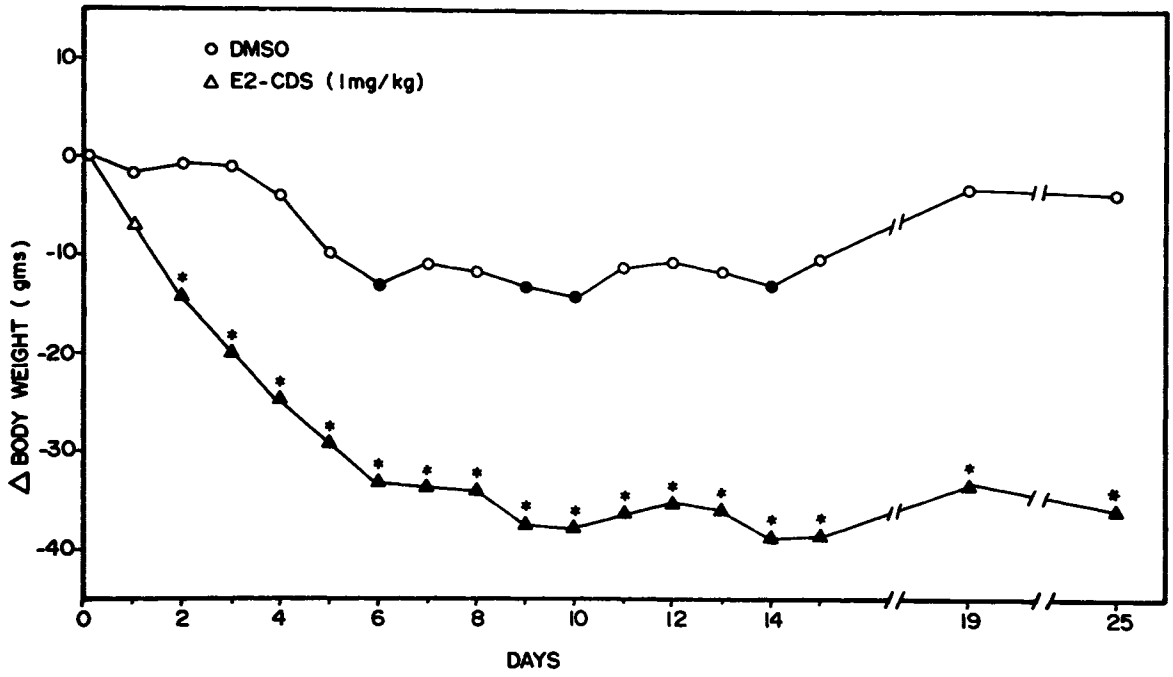

Fig. 6. Effects of E_2-CDS on body weight in ovariectomized rats. Animals received a single intravenous injection of the vehicle (DMSO; O——O) or E_2-CDS (1.0 mg/kg; Δ——Δ). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values and asterisks indicate significant differences ($P < 0.05$) from the DMSO group at the same sampling time. Initial (Day 0) body weights did not differ significantly between groups and data for each rat were normalized to its initial body weight. The significance of differences between mean values over time was determined by ANOVA and SNK tests. Differences between groups at particular sampling times was determined by Student's t tests. $N = 7$ rats per group.

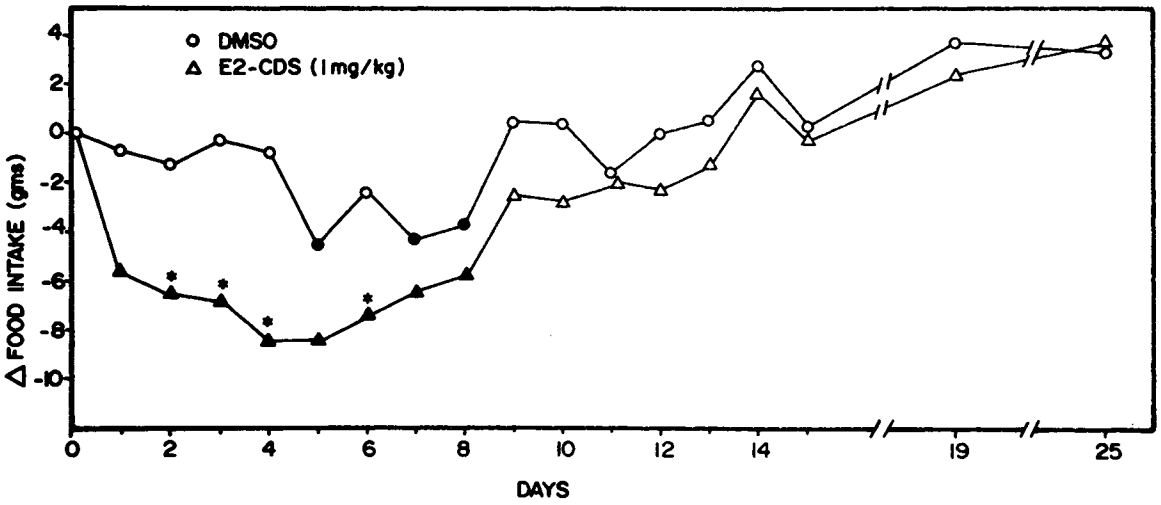

Fig. 7. Effects of E_2-CDS on daily food intake in ovariectomized rats. Animals received a single intravenous injection of the vehicle (DMSO; ○——○) or E_2-CDS (1.0 mg/kg; △——△). Filled symbols indicate significant differences ($P < 0.05$) from the preinjection (Day 0) values and asterisks indicate significant differences ($P < 0.05$) from the DMSO group at the same sampling time. Initial (Day 0) food intake values did not differ significantly between groups and data for each rat were normalized to its initial food intake level. The significance of differences between mean values over time was determined by ANOVA and SNK tests. Differences between groups at particular sampling times was determined by Student's t tests. $N = 7$ rats per group. The data depicted here are from the animals whose body-weight response is shown in Fig. 6.

one, the latter phase of body-weight gain is absent in response to E_2-CDS in OVX rats. The possibility that changing levels of brain E_2 after administration of E_2-CDS may contribute to the shift from weight loss to weight gain is unlikely inasmuch as we have observed that brain levels of E_2 exceed 3.5 ng/g tissue through the time course of the present study after an E_2-CDS dose of 1 mg/kg (Rahimy *et al.*, unpublished observations). These levels of brain E_2 are far in excess of those needed to suppress body weight.

The transient phase of food intake suppression observed presently after a single iv injection of E_2-CDS and previously reported for systemic administration of E_2 (1,2) is also observed following the local implantation of E_2 into brain regions (17–19). It appears that the effects of E_2 on food intake are, in part, centrally mediated and evidence has been reported for the central mediation of other responses to E_2 which would affect body weight. Thus, E_2 has been shown to increase locomotor activity (1,2,35,36), heat production (5), and lipoprotein lipase activity (18) following systemic or local implantation into the brain. This central effect of E_2 after E_2-CDS administration does not appear to be the result of alterations in the secretion of anterior pituitary hormones since we have observed that the secretions of growth hormone, thyroid stimulating hormone, thyroxine, and triiodothyronine are not altered after E_2-CDS administration (30). Further, weight loss is observed in OVX rats at doses of E_2-CDS which do not elevate serum prolactin levels (37). It should be pointed out, however, that evidence exists which suggests that a component of the weight-reducing effects of E_2 is mediated by peripheral mechanisms (2).

In summary, we have observed a biphasic effect of a novel chemical delivery system for the brain-enhanced delivery of estradiol on body weight in female rats. The profile of the response is ovarian dependent in that OVX rats exhibit a chronic weight loss without compensatory increases in body weight or food intake.

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