Research Article

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

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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 (E2-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, E2-CDS (0.2, 1.0, or 5.0 mg/kg), equimolar doses of another 17-hydroxy-substituted estrogen, estradiol valerate (E2-VAL), or the dimethyl sulfoxide (DMSO) vehicle to female rats. Daily food intake and body weight was determined for 24 days thereafter. E2-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 E2-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, E2-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 E2-CDS, long-term ovariectomized rats were treated with E2-CDS (1.0 mg/kg) or the vehicle and parameters of body weight regulation were determined for 25 days. Ovariectomized rats responded to E2-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 E2-CDS on the day of diestrus 1, 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 E2 to the brain with E2-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 (E2) 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 E2 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 E2 (1,2,14–16) but not progesterone (1,2). E2 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 E2. Additionally, localized lesions of hypothalamic and septal regions can reduce or blunt the inhibitory effects of E2 on body-weight gain in ovariectomized rats (1,2).

That E2 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 E2 in body-weight regulation in a variety of species, the potential for the therapeutic use of estrogens to reduce body
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 dihydroxyproline moiety to the drug and the in vivo oxidation of the dihydroxyproline to a pyridinium ion (21). The lipophilic dihydroxyproline 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 (E2-CDS) as a probe for separating centrally mediated from peripherally mediated effects of estradiol, we undertook an evaluation of the effects of E2-CDS on body weight and food intake in female rats.

MATERIALS AND METHODS

Drug Synthesis and Administration

The synthesis of the E2-CDS has been described by us in detail (22–24). Briefly, the 2,17β-dinicotinate ester of E2 was made by refluxing 17β-E2 with nicotinoyl chloride or nicotinic anhydride in hydrazine. This derivative was selectively hydrolyzed to the 17-monoester of E2 with potassium bicarbonate in 95% methanol. The monoester of E2 was then quaternized with methyl iodide. The delivery system was then prepared by reduction of the monoester of E2 with Na2S2O4. The structure of each intermediate and final product was confirmed by nuclear magnetic resonance and elemental analysis: mp 115–130°C dec; NMR (CDCl3) δ 7.0–6.8 (m, 2H, C-1 E2 proton + C-2 pyridine H), 5.0–4.5 (m, 3H, C-17β E2 + C-5 pyridine + phenolic OH, exchangeable), 3.2–3.0 (m, 2H, C-4 pyridine protons), 3.0–2.9 (s, 3H, NCH3), 2.9–1.1 (m, 15H, E2 skeletal H’s), 1.0–0.9 (s, 3H, C-18 E2 protons). The yields at each synthetic step were 64–94%.

Estradiol valerate (E2-VAL) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Both drugs were dissolved in dimethyl sulfoxide (DMSO). E2-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. E2-VAL was diluted in DMSO to achieve doses which were equimolar to that of E2-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 E2-CDS are well described with this and no other routes of administration (22–24). E2-VAL was chosen as a positive control for comparison with E2-CDS since it, like E2-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 E2-CDS/kg body wt or equimolar doses of E2-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 E2-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.