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Estradiol acts in the medial preoptic area, arcuate nucleus, and dorsal raphe nucleus to reduce food intake in ovariectomized rats

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ABSTRACT

Estradiol (E2) exerts an inhibitory effect on food intake in a variety of species. While compelling evidence indicates that central, rather than peripheral, estrogen receptors (ERs) mediate this effect, the exact brain regions involved have yet to be conclusively identified. In order to identify brain regions that are sufficient for E2's anorectic effect, food intake was monitored for 48 h following acute, unilateral, microinfusions of vehicle and two doses (0.25 and 2.5 μg) of a water-soluble form of E2 in multiple brain regions within the hypothalamus and midbrain of ovariectomized rats. Dose-related decreases in 24-h food intake were observed following E2 administration in the medial preoptic area (MPOA), arcuate nucleus (ARC), and dorsal raphe nucleus (DRN). Within the former two brain areas, the larger dose of E2 also decreased 4-h food intake. Food intake was not influenced, however, by similar E2 administration in the paraventricular nucleus, lateral hypothalamus, or ventromedial nucleus. These data suggest that E2-responsive neurons within the MPOA, ARC, and DRN participate in the estrogenic control of food intake and provide specific brain areas for future investigations of the cellular mechanism underlying estradiol's anorexigenic effect.

Introduction

Behavioral studies in rats reveal that estradiol's (E2's) anorexigenic effect occurs slowly, within hours to days following acute, peripheral E2 treatment (Geary and Asarian, 1999; Asarian and Geary, 2002). This has prompted investigations of the relative contributions of the two nuclear estrogen receptor (ER) subtypes, ERα and ERβ, to the estrogenic control of food intake. Studies involving ER null mice and pharmacological manipulations of ERα and ERβ suggest that activation of ERα is both sufficient and necessary for E2's anorexigenic effect (Geary et al., 2001; Roesch, 2006; Santollo et al., 2007; Thammacharoen et al., 2009; Santollo et al., 2010). Less is known, however, regarding the sites of the critical ERs. While E2 is released by the ovaries, thereby gaining easy access to peripheral ERs, it also readily crosses the blood-brain barrier, thereby gaining access to central ERs. As such, E2's anorexigenic effect could be mediated by activation of peripheral and/or central ERs.

The relative contributions of peripheral and central ERs to E2's anorexigenic effect have been addressed in three studies involving ICI 182,780, an ER antagonist with limited ability to cross the blood-brain barrier. In the earlier studies, peripheral administration of ICI 182,780 failed to influence the anorexigenic effect of chronic E2 treatment in ovariectomized (OVX) rats and hamsters (Wade et al., 1993a; Wade et al., 1993b). Recently, we extended these reports by demonstrating that peripheral administration of ICI 182,780 fails to alter the anorexia following acute E2 treatment in OVX rats. Peripheral administration of ICI 182,780 did, however, block E2's proliferative effect on uterine tissue, thereby confirming extensive blockade of peripheral ERs (Rivera and Eckel, 2010). In comparison, central administration of ICI 182,780 was sufficient to block E2's acute, anorexigenic effect in OVX rats. Our observation that E2 continued to induce vaginal estrus in these same rats confirms that centrally administered ICI-182,780 did not leak into the periphery (Rivera and Eckel, 2010). Taken together, these studies demonstrate that activation of central, rather than peripheral, ERs is necessary for E2's anorexigenic effect.

It is likely that E2's anorexigenic effect is mediated by a distributed neuronal network since E2 influences the activity of a number of hypothalamic neuropeptide and neurotransmitter systems implicated in the control of food intake. For example, studies in rodents reveal that estradiol decreases neuropeptide Y (NPY) and melanin-concentrating hormone (MCH) mRNA expression in the arcuate nucleus (ARC) and the lateral hypothalamus (LH), respectively (Baskin et al., 1995; Murray et al., 2000; Pelletier et al., 2007). In addition, estradiol increases pro-opiomelanocortin (POMC) and corticotropin-releasing factor (CRF) mRNA expression in the ARC and paraventricular nucleus of the hypothalamus (PVN), respectively (Pelletier et al., 2007). Thus, it is likely that the critical, central ERs underlying E2's anorexigenic effect are located in multiple brain areas.

One approach to identify the locations of the critical ERs involves direct administration of E2 in specific brain areas. Of the hypothalamic nuclei, the PVN has received the most attention. Butera and colleagues were the first to report that administration of dilute, crystalline E2 in

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the PVN decreased food intake in OVX rats and guinea pigs (Butera and Czaja, 1984; Butera and Beikirch, 1989) and that subcutaneous administration of E2 failed to decrease food intake in OVX rats with bilateral lesions of the PVN (Butera et al., 1992). Because others have failed to replicate these findings (Dagnault and Richard, 1994; Hrupka et al., 2002), the role of the PVN in the estrogenic control of food intake remains unresolved. More recently, the involvement of additional hypothalamic and hindbrain sites have been examined. Similar to the PVN, there are conflicting reports regarding the medial preoptic area's (MPOA's) role in the estrogenic control of food intake. Richard and colleagues reported dose-related decreases in food intake following microinfusions of a water-soluble form of E2 in the MPOA (Dagnault and Richard, 1997), whereas Geary and colleagues were unable to replicate these findings when placing dilute crystalline E2 implants in the MPOA (Hrupka et al., 2002). One report exists that direct administration of E2 in the nucleus of the solitary tract is sufficient to decrease food intake in OVX rats (Thammacharoen et al., 2008). Finally, there has been agreement to date that the ventromedial hypothalamus (VMH) is not involved in the estrogenic control of food intake. Implants of both pure and dilute crystalline E2 in the VMH fail to reduce food intake (Palmer and Gray, 1986; Butera and Beikirch, 1989) and rats with VMH lesions display a reduction in food intake following E2 treatment (King and Cox, 1973).

Although the available literature suggests that E2 acts in the brain to decrease food intake, methodological differences among previous studies may have contributed to the equivocal findings regarding the sites of the critical ERs. Studies attempting to replicate previous findings often used different forms of E2 (i.e. crystalline or water-soluble) that may have resulted in differing concentrations of E2 in the brain. Other methodological inconsistencies across studies involve the duration of E2 treatment (acute versus chronic application), the type of E2 (E2 benzoate, 17-β E2, water-soluble E2), and differing rat strains (Long Evans, Wistar, Sprague–Dawley). The present study was designed to address some of these methodological inconsistencies by examining food intake in a single strain of OVX rats receiving similar site-specific, unilateral microinfusions of a water-soluble form of E2 in various hypothalamic brain areas. The small volume and lipophobic properties of this form of E2 were chosen to minimize the spread of E2. In addition, acute hormone treatment was used in an attempt to more accurately model the cyclic rise in endogenous E2 secretion in the intact female rat and to prevent a sustained elevation in central E2 concentration.

The goal of this experiment was to target multiple brain areas using a unified approach involving acute E2 administration to identify nuclei sufficient for E2's anorexigenic effect. The lateral ventricle was included as a positive control and to establish an upper boundary of E2 doses to be administered in specific brain regions. The PVN and MPOA were chosen on the basis of at least one positive report that site-specific administration of E2 within these brain areas reduced food intake in OVX rats (Butera and Beikirch, 1989; Dagnault and Richard, 1997). The ARC, LH, and DRN were chosen because they each synthesize either a neuropeptide or a neurotransmitter (e.g., NPY, MCH, and serotonin respectively) that has been implicated in the estrogenic control of food intake (Rivera and Eckel, 2005; Eckel et al., 2005; Messina et al., 2006; Santollo and Eckel, 2008a; Santollo and Eckel, 2008b). The VMH was chosen as a negative control region because it is well established that E2 does not act in the VMH to decrease food intake (Palmer and Gray, 1986; Butera and Beikirch, 1989).

Materials and methods

Animals and housing

Female, Long-Evans rats (Charles River Breeding Laboratory, Raleigh, NC) weighing 225–250 g at study onset, were housed individually in custom-designed cages. Each cage was equipped with a feeding niche that provided access to a spill-resistant food cup. Rats were given free access to powdered rat chow (Purina 5001) and tap water, except where otherwise noted. The testing rooms were maintained at 20 ± 2 °C under a reverse 12:12-h light–dark cycle (dark onset = 1300 h). Animal usage and all procedures were approved by the Florida State University Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized by intraperitoneal (i.p.) injections of a mixture of ketamine (50 mg/kg; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (4.5 mg/ml; Rompun, Mobay, Shawnee, KS) and then bilaterally OVX using an intra-abdominal approach. Immediately following ovariectomy surgery, rats were implanted with single, stainless-steel, guide cannulae (26-g (nucleus-specific) or 22-g (lateral ventricle), Plastics One, Roanoke, VA) targeting the MPOA (AP: −0.4 mm; ML −0.5 mm; DV: −7.5 mm; $n = 12$), ARC (AP: −3.5 mm; ML: −0.4 mm; DV: −9.3 mm; $n = 10$), DRN (AP: −7.8 mm; ML: −4.4 mm; DV: −7.2 mm; 35° angle; $n = 13$), PVN (AP: −1.8 mm; ML: −0.4 mm; DV: −7.4 mm; $n = 10$) , LH (AP: −2.6 mm; ML: −1.1 mm; DV: −8.0 mm; $n = 9$), VMH (AP: −2.6 mm; ML: −0.7 mm; DV: −9.0 mm; $n = 7.5$), or lateral ventricle (AP: −0.6 mm; ML: −1.7 mm; DV: −3.5 mm; $n = 18$). The coordinates were chosen in an attempt to target the portion of each brain area containing the greatest density of ERα expression (Jacobs and Azmitia, 1992; Yokosuka et al., 1997; Sato et al., 2005; Muschamp and Hull, 2007). The depths of the cannulae were chosen to target the upper border of the brain area of interest in order to limit tissue damage within targeted brain areas. Following surgery, rats received i.p. injections of butorphanol (0.5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and gentamicin (10 mg/ml; Pro Labs Ltd, St. Joseph, MO) to minimize post-surgical pain and the risk of infection, respectively. Behavioral testing did not commence until food intake and body weight returned to pre-surgical levels (~7 days).

Cannula verification

Ventricular cannulae were verified following post-operative recovery by monitoring light-phase water intake following intracerebroventricular (i.c.v.) microinfusion of 50 ng of angiotensin II (Sigma-Aldrich, St. Louis, MO), delivered in 5 μl of saline vehicle over a period of 1 min. Only those rats that consumed at least 5 ml of water in 20 min were included in the study. All but three rats passed this criterion (15 of 18 placements were correct; mean consumption = 8.8 ± 1.2 ml).

Nuclei-specific cannulae were verified using postmortem histology. At the end of the study, rats were anesthetized with 0.5 ml of sodium pentobarbital (50 mg/ml Henry Schein, Melville, NY) and, once unresponsive, decapitated. Their brains were removed and stored in 10% formalin (Sigma-Aldrich, St. Louis, MO) for 48 h and then stored in a 30% sucrose solution for cryoprotection. One week later, brains were sectioned on a cryostat (Ziess) at 40 μm intervals. Serial sections were obtained through the nuclei of interest and then stained with cresyl violet (Sigma-Aldrich). A rater, unaware of the behavioral data, assessed cannula placements in accordance with a rat stereotaxic atlas (Paxinos and Watson, 1998). Rats in which the tips of the cannulae were misplaced (greater than 300 μm beyond the targeted area in any direction, i.e. dorsal, ventral, rostral or caudal) were excluded from data analysis. This resulted in 39 rats being included in the data analysis; 7 out of 12 MPOA, 6 out of 10 ARC, 6 out of 13 DRN, 7 out of 10 PVN, 6 out of 9 LH, and 7 out of 12 VMH (Fig. 1).