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Cyclic estradiol treatment modulates the orexigenic effects of ghrelin in ovariectomized rats

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ABSTRACT

Data from a wide variety of mammalian species indicate that feeding behavior can be influenced by changes in endogenous estrogens and exogenous estrogenic treatments. Ghrelin is an important physiological signal for the regulation of energy balance, and ghrelin treatment increases eating and body weight in male rodents. The following studies evaluated the hypothesis that the inhibitory effects of estradiol on feeding involve interactions with orexigenic peptides by examining the ability of estradiol to modulate the behavioral effects of ghrelin in female rats. In these experiments, adult rats were ovariectomized and assigned to an estradiol benzoate (EB) or an oil (control) group. Three weeks after ovariectomy, animals received two daily subcutaneous injections of EB or the oil vehicle. Animals then received intraperitoneal (ip) injections of ghrelin (6.0 or 12.0 nmol) or saline during the nocturnal and diurnal periods three days after the first injection of estradiol or oil. Food intake, meal size, and meal number were determined during the 2-hour period following ghrelin or saline treatments. Ghrelin significantly increased food intake during nocturnal tests in oil-treated but not estradiol-treated rats. The hyperphagic effects of ghrelin on nocturnal food intake were also accompanied by an increase in meal size, and this effect of ghrelin on meal size was attenuated in estradiol-treated females. These findings support the hypothesis that the effects of estradiol on feeding behavior involve an attenuation of orexigenic signals, possibly by modulating the effects of the peripheral ghrelin signal on hypothalamic neuropeptides involved in the control of food intake.

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1. Introduction

Apart from their well-known effects on reproductive physiology and behavior, ovarian hormones also play an important role in the control of feeding behavior in female mammals. For example, food intake fluctuates across the ovarian cycle in rats and guinea pigs, with a decline in daily food intake seen during behavioral estrus, which occurs after the rise in plasma estradiol (Blaustein and Wade, 1976; Czaja and Goy, 1975; ter Haar, 1972). In women, caloric intake is reduced during the follicular phase of the menstrual cycle, following the rise in estradiol levels prior to ovulation (Gong et al., 1989; Lyons et al., 1989). Thus, food intake is significantly reduced at a point in estrous and menstrual cycles when estradiol levels have been increasing. Although the relationship between ovarian hormones and ingestive behavior has been studied in a wide range of mammalian species, estradiol's effects on feeding behavior and body weight have been best characterized in female rats. For example, withdrawal of estradiol via ovariectomy causes a rapid increase in food intake, weight gain, and adiposity (Asarian and Geary, 2002; Blaustein and Wade, 1976; Holt et al., 1936; Wade, 1975). These changes in eating and body weight seen in ovariectomized (OVX) rats can be reversed by peripheral treatment with physiological doses of estradiol (Asarian and Geary, 2002; Wade, 1975).

Although these effects of estradiol on food intake appear to be mediated in part by interactions with cholecystokinin (CCK) systems that participate in the control of satiety and meal size (Asarian and Geary, 1999; Butera et al., 1993; Geary et al., 1994), the observation that CCK antagonists do not completely reverse the anorectic action of estradiol indicates that CCK is not the only factor involved in mediating estrogenic effects on feeding (Eckel and Geary, 1999). Along these lines, research conducted in our lab and by other investigators has focused on the ability of estradiol to attenuate the effects of orexigenic peptides like ghrelin. Discovered in 1999 as an endogenous ligand for the growth hormone secretagogue receptor (Kojima et al., 1999), ghrelin is a peptide produced by the stomach that is best known for its effects on hunger (Cummings et al., 2001). Fasting increases ghrelin levels in rodents and humans whereas eating suppresses ghrelin secretion (Beck et al., 2002). Administration of ghrelin also increases eating in animals and humans (Kojima et al., 1999; van der Leley et al., 2004),

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and peripheral or central administration of antibodies to ghrelin inhibits food intake in rats (Bagnasco et al., 2003; Nakazato et al., 2001). Although the studies evaluating the effects of ghrelin on feeding cited above used male rodents and humans, research indicates that there are sex differences in the orexigenic action of ghrelin. Intact male rats and OVX female rats are more responsive to the stimulatory effects of ghrelin on feeding than intact females. In addition, this sex difference appeared to be mediated by estradiol, as the effects of ghrelin on food intake were attenuated in OVX rats treated with estradiol relative to oil-treated controls (Butera, 2010; Clegg et al., 2007).

Whereas the orexigenic effects of ghrelin have been well documented, few studies have examined how ghrelin affects spontaneous meal patterns, and those findings have been mixed. For example, Azzara et al. (2005) found that ip injections of ghrelin significantly increased nocturnal food intake and meal size in male mice. Ghrelin had no significant effects on meal frequency in that experiment. By contrast, Clegg et al. (2007) showed that estradiol decreased the orexigenic effect of ghrelin by an attenuation of ghrelin's ability to decrease the latency to eat during diurnal feeding tests. This finding was unexpected, as it differs from the well-characterized behavioral mechanism by which estradiol decreases food intake, which is through a selective decrease in meal size.

In the following experiments, OVX rats were treated with a cyclic regimen of estradiol replacement (or the oil vehicle) and changes in food intake, meal size, and meal number were measured following intraperitoneal injections of ghrelin. In addition, the effects of ghrelin on both nocturnal and diurnal food intake were assessed in order to determine whether the orexigenic effects of ghrelin are modulated by estradiol and/or the time of day when the peptide is given. Therefore, one of the goals of the present study was to provide a more comprehensive analysis of the effects of ghrelin on meal patterns during both the diurnal and nocturnal periods in order to address some of the discrepancies described above. It is hypothesized that ghrelin will increase food intake in oil-treated animals and that this orexigenic effect of ghrelin will be attenuated by estradiol. These planned comparisons will be incorporated into the data analyses.

2. Materials and methods

2.1. Animals, housing, and ovariectomy

Twenty female Long–Evans rats obtained from Taconic Farms, Inc. (Hudson, NY) served as subjects in these experiments. Animals were approximately 50 days of age upon arrival and were housed in pairs in Plexiglas cages (10 1/2″ × 19″ × 8″) in a windowless colony room. The room was maintained at 21 ± 3 °C with a 12:12 h light–dark cycle (lights on at 0400 h). A white noise generator (Lafayette Instruments, Lafayette, IN) was used to mask any outside noise. Pelleted rodent chow (Mazuri, Brentwood, MO) and tap water were available ad libitum. Following the end of the experiment, animals were euthanized by ip injections of sodium pentobarbital (65 mg/kg; FatalPlus, Vortech Pharmaceuticals, Dearborn, MI).

Following a one-week acclimation period to their new environment, the rats were ovariectomized via a single midline abdominal incision under ketamine (75 mg/kg; Ketaset, Fort Dodge, IA) and xylazine (5 mg/kg, Xyla-ject, Phoenix Scientific, St. Joseph, MO) anesthesia via ip injections. Each ovary and each respective uterine horn were located, the uterus tied off, and the ovary in addition to approximately 1 cm of the uterus was excised. The incision was then closed with 4 sutures. Animals were given a subcutaneous injection of butorphanol (0.5 mg/kg, Torbugesic, Fort Dodge, IA) after surgery as an analgesic. Ten days after surgery, animals were transferred to the test cages described below. All procedures were approved by the Niagara University Institutional Animal Care and Use Committee and were consistent with the ethical standards of the American Psychological Association.

2.2. Spontaneous meal patterns

Animals were individually housed in Plexiglas cages (43.2 × 43.2 × 19 cm) equipped with computer-controlled food dispensers (Med Associates, St. Albans, VT). Rodent food pellets (45 mg, NOYES Precision Pellets, Research Diets, Inc., New Brunswick, NJ) were dispensed in response to the activation of a photosensor placed above each food cup. Animals were allowed free access to food throughout the study. Tap water was provided by a water bottle adjacent to the food cup and refilled daily. The Plexiglas cages were connected via an interface (Med Associates, St. Albans, VT) to an IBM-compatible computer. The software provided with this system (MED-PC IV) records the number of pellets dispensed and converts the data into spontaneous feeding patterns (e.g., time and bouts of feeding, amount consumed during each interval). A meal was defined as any feeding bout of at least 0.2 g that is separated from other feeding bouts by at least 15 min. Using these criteria, recorded meals account for 96% of daily food intake in female rats (Eckel et al., 1998). The data collected were saved to disk for subsequent analyses. The raw data were converted into Excel files using MED-PC 2 Excel and translated into meal size and meal number with the Tongue Twister program (T.A. Houpt, Florida State University, Tallahassee, FL) on a Macintosh G4 computer. Animals were given a 10-day acclimation period in the test cages with the new diet before the onset of hormone treatments and data collection.

2.3. Treatment protocol

For the diurnal tests, the MED-PC system was shut down at 0900 h each day for maintenance and data collection. During this time animals were weighed to the nearest gram on an electronic balance and water bottles were refilled. The MED-PC system was then restarted at 10:00 h.

Following the acclimation period described above, animals were matched for body weight and assigned to one of two groups: estradiol benzoate (EB, 5.0 μg, n = 10) and oil (control, n = 10). This was done to insure that there were no pre-existing differences in body weight between the estradiol and control groups prior to the start of the experiment. Previous findings in our lab indicate that this dose of EB can suppress food intake and meal size in OVX rats (Butera et al., 2010). Animals received subcutaneous injections of 5.0 μg EB (Sigma, St. Louis, MO, dissolved in 0.1 ml sesame oil) or the oil vehicle (0.1 ml) at 0930 for two days. This cyclic pattern of estradiol replacement produces behavioral and neurochemical changes associated with estrus without neuroendocrine and behavioral carryover effects that accompany continuous hormone replacement paradigms (Schumacher et al., 1991). Three days after the first EB or oil injection, the animals were given an intraperitoneal (ip) injection of either 6.0 or 12.0 nmol of octanoylated rat ghrelin (Bachem, Temecula, CA) dissolved in 0.9% endotoxin-free saline or the saline vehicle (0.1 ml). The doses of ghrelin were chosen based on our preliminary findings and a previous report showing that they increase food intake in male rats (Davidson et al., 2005). The injections were administered at 0950 h and food intake, meal size, and meal number were determined for the subsequent 2-hour period following the restart of the MED-PC program at 10:00 h.

For the nocturnal tests, the Med-PC system was turned off at 14:00 h for cleaning, maintenance, and data collection as described above, and the MED-PC system was then restarted at 15:00 h. Animals received subcutaneous injections of 5.0 μg EB or the oil vehicle (0.1 ml) at 14:30 for two days. Three days after the first EB or oil injection, the animals were given an intraperitoneal (ip) injection of either 6.0 or 12.0 nmol of octanoylated rat ghrelin dissolved in 0.9% endotoxin-free saline or the saline vehicle (0.1 ml). The injections were administered at 14:50 h (just before the onset of the nocturnal period) and food intake, meal size, and meal number were determined for the subsequent 2-hour period following the restart of the MED-PC program at 15:00 h. The procedure was repeated each week until all animals were tested with saline and both doses of ghrelin during the diurnal and nocturnal

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