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Effects of galanin-like peptide (GALP) on locomotion, reproduction, and body weight in female and male mice

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Abstract

Galanin-like peptide (GALP) has been implicated in the neuroendocrine regulation of both feeding and reproduction. In male rodents and primates, intracerebroventricular (icv) infusions of GALP stimulate luteinizing hormone (LH) release, induce Fos expression in brain areas implicated in feeding and reproduction, and affect food intake and body weight in rodents. In gonad-intact and castrated male rats, icv administration of GALP also stimulates male sexual behavior. While the effects of GALP on male physiology and behavior are well documented, no studies have addressed such a role of GALP in females. We tested the effects of icv GALP infusions on LH release, locomotor activity, motor control, and body weight regulation in adult ovariectomized female mice hormonally primed with estradiol benzoate and progesterone. In addition, sexually-experienced male and female mice were treated with GALP and tested for sexual behavior. In females, GALP reduced open-field locomotor activity, the ability to maintain grip on an accelerating rotarod, and 24-h body weight in a dose-dependent manner. GALP also increased LH secretion in female mice, an effect that was blocked by pre-treatment with Antide, a gonadotropin-releasing hormone (GnRH) type-1 receptor antagonist. GALP infusions slightly decreased the occurrence of lordosis behavior in female mice and significantly increased the latencies with which females displayed receptivity. Unlike previous reports in male rats, GALP inhibited male sexual behavior in mice. Our data indicate that in female mice, GALP stimulates LH release via GnRH, and decreases body weight, motor control, and locomotor activity via GnRH-independent pathways. Furthermore, our sexual behavior and locomotor findings suggest species-specific differences in the mechanism and/or location of GALP action in the brains of rats and mice.

Keywords: Sexual behavior; Energy balance; Motor control; Coordination; Exploration; Luteinizing hormone; Gonadotropin-releasing hormone

Introduction

Galanin-like peptide (GALP) is a 60-amino acid neuropeptide of which amino acids 9–21 share 100% sequence identity with the first 13 amino acids of the neuropeptide galanin (Cunningham, 2004; Ohtaki et al., 1999). GALP was initially cloned from the pig, human, and rat, and cDNAs from non-human primates and mice have recently been characterized (Cunningham et al., 2002; Jureus et al., 2001; Ohtaki et al., 1999). In all species examined, GALP and galanin are encoded by distinct genes (Cunningham, 2004). In contrast to galanin, which is widely distributed throughout the brain, the expression of GALP within the mammalian central nervous system is exclusively localized to the hypothalamic arcuate nucleus, the median eminence, and the infundibular stalk (Jureus et al., 2001; Kerr et al., 2000; Larm and Gundlach, 2000; Takatsu et al., 2001). The highly localized distribution of hypothalamic cells expressing GALP mRNA is well conserved across mammalian species (Cunningham, 2004; Cunningham et al., 2002; Jureus et al., 2001). Fibers expressing GALP immunoreactivity are located in several forebrain regions implicated in the regulation of feeding, energy balance, and/or reproduction including the paraventricular nucleus (PVN), medial preoptic area (mPOA), bed nucleus of the stria

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terminalis (BNST), periventricular nucleus, and the arcuate nucleus (Takatsu et al., 2001).

The arcuate nucleus, where most GALP-containing neurons are located, plays a critical role in the regulation of mammalian energy balance and reproduction (Gottsch et al., 2004), and several discrete populations of neurons in this nucleus are targets for the regulatory hormone leptin (McMinn et al., 1998, 2000). Furthermore, in both rodents and primates, the arcuate nucleus contains mRNA of numerous regulatory neuropeptides involved in feeding and/or reproduction including neuropeptide Y (NPY), α-melanocyte-stimulating hormone (α-MSH), agouti-related protein (AgRP), and galanin (Cone et al., 2001). Immunocytochemistry and in situ hybridization analyses have revealed that most GALP neurons express leptin receptors (Cunningham et al., 2002; Jureus et al., 2000; Takatsu et al., 2001) and as many as 40% express the NPY Y1 receptor (Cunningham et al., 2004). Collectively, these data suggest that GALP has a critical role in the regulation of energy balance and/or reproduction. Although central infusions of GALP stimulate acute feeding (1 h) in rats (Lawrence et al., 2002; Matsumoto et al., 2002; Seth et al., 2003), other studies in mice and rats have reported decreases in long-term (14 h, 24 h) food intake and a simultaneous reduction in body weight following GALP treatment (Hansen et al., 2003; Krasnow et al., 2003, 2004; Lawrence et al., 2002). Interestingly, male mice also display decreased locomotor activity for several hours after GALP infusion (Krasnow et al., 2003), an effect not observed in rats (Cunningham, 2004).

In addition to their effects on feeding, GALP infusions also significantly influence male reproductive physiology and behavior. GALP infusions stimulate luteinizing hormone (LH) release in a dose-dependent manner in male rats, mice, and macaques (Cunningham et al., 2004; Krasnow et al., 2003, 2004; Matsumoto et al., 2001). This GALP-induced LH release is likely mediated via GnRH I-dependent pathways, as GnRH I receptor antagonists prevent GALP from stimulating LH release in rats and primates (Cunningham et al., 2004; Matsumoto et al., 2001). Furthermore, GALP can cause in vitro release of GnRH from hypothalamic explants and GALP infusions also increase Fos immunoreactivity in GnRH neurons (Matsumoto et al., 2001; Seth et al., 2004). Recently, Fraley et al. (2004) reported increased male sexual behavior in gonad-intact and castrated rats infused with GALP, indicating that GALP's effects on reproduction also include the regulation of behavior.

Interestingly, while GALP's regulatory function in males has been well studied, no studies have addressed the physiological and behavioral roles of GALP in females of any mammalian species. We therefore conducted several studies in female mice to elucidate the role of GALP in reproduction, locomotion, and energy balance. Specifically, we addressed the following critical questions: (1) Does GALP effect LH release and body weight in female mice, and if so, are these effects mediated by GnRH-dependent pathways? (2) Are GALP's locomotor effects previously reported in male mice also present in females, and if so, what is the underlying basis for the reduced activity? (3) Does GALP stimulate sexual behavior in mice of either sex as it does in male rats?

Methods and materials

Subjects

All experiments used adult (2–4 months of age), initially sexually-naïve male and female C57BL/6J mice purchased from the Jackson Laboratory (Bar Harbor, ME). Female mice were group housed (2–3 per cage) with food (Harlan Diet 8604; Harlan Teklad, Madison, WI) and water available ad libitum. After cannula implantation, female mice were individually housed. Male mice were singly housed for the entire duration of the study. The room was maintained on a 12-h light, 12-h dark photoperiod (lights off at 1200 h) at a temperature of 23 ± 2°C. All behavior tests were conducted during the dark portion of the light cycle (1–5 h after lights out) under constant red-light illumination. All procedures were approved by the Animal Care and Use Committee of the University of Virginia.

Gonadectomies and stereotaxic implantation of cannula

Ovariectomies and castrations were conducted under general anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine injected ip) 2–4 weeks prior to the beginning of the study. Females were at least 8 weeks old and males at least 10 weeks old when the gonadectomies were performed. At the time of castrations, males were simultaneously implanted with a subcutaneous Silastic implant containing testosterone (5 mm long; outer diameter 2.2 mm, inner diameter 1.0 mm).

To implant intracerebroventricular (icv) cannulas, mice were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine injected ip) and fit into a mouse stereotaxic apparatus (Kopf Instruments, Tujunga, CA). A mid-line incision was made along the top of the head, exposing bregma; guide cannulas (26 gauge; Plastics One, Roanoke, VA) containing an internal dummy cannula were centered on bregma and moved −0.2 mm rostral caudal and −1.0 mm medial lateral. A hole was then drilled in the skull and the cannula was lowered to a depth of 2.8 mm, aimed at the lateral ventricle. The cannula was fixed to the skull with glue and dental acrylic. Mice were then given an analgesic (ketoprofen, sc; 2 mg/kg) and kept warm on a heating pad until regaining consciousness.

Peptide infusions

For icv infusions, mice were briefly anesthetized with isoflurane inhalant and infused with 5 μl of either vehicle (0.09% saline), GALP, GNRH-I, or Antide (see specific