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Galanin-like peptide stimulates feeding and sexual behavior via dopaminergic fibers within the medial preoptic area of adult male rats

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ABSTRACT

Galanin-like peptide (GALP) is located in the arcuate nucleus (Arc) of the hypothalamus and is known to regulate both food intake and sexual behaviors in adult male rats. We have previously demonstrated that ICV GALP administration elicits a significant fos response within the medial preoptic area (mPOA). GALP is known to stimulate both food intake and male-typical sex behavior, presumably by direct actions within the mPOA. Recent data from our and other labs have led us to suspect that GALP effects on sex behaviors are due to activation of incertohypothalamic dopaminergic neurons that terminate within the mPOA. To test the hypothesis that GALP activates mPOA dopaminergic systems, we utilized an immunolesion technique to eliminate dopaminergic fiber input to the mPOA via a dopamine transporter-specific toxin (DATSAP, $n = 8$) and compared to control injections (SAP, $n = 8$). All animals were sexually experienced adult male Long-Evans rats. DATSAP-treated male rats showed a significant ($p < 0.001$) reduction in male sexual behaviors compared to SAP controls. We found that elimination of dopaminergic fibers within the mPOA significantly ($p < 0.001$) eliminated all aspects of male sexual behavior under normal mating paradigms. Injections of GALP (5.0 nmol) significantly increased ($p < 0.01$) male sex behavior and food intake in SAP control male rats but GALP did not stimulate the expression of these behaviors in DATSAP-treated rats. The orexigenic and anorexigenic effects of GALP were significantly ($p < 0.001$) attenuated in DATSAP-treated male rats compared to SAP controls; however, ICV GALP was still able to significantly ($p < 0.05$) reduce 24 h body weight in both DATSAP and SAP rats. ICV GALP significantly ($p < 0.05$) stimulated fos within the mPOA of SAP rats but not in DATSAP-treated male rats. These data suggest that GALP activates feeding and sexual behaviors in male rats by stimulating dopaminergic neurons that terminate within the mPOA.

1. Introduction

Galanin-like peptide (GALP), originally isolated from the porcine hypothalamus, is a 60-amino acid neuropeptide that shares a partial homology with galanin (Ohtaki et al., 1999; Kerr et al., 2000; Jureus et al., 2001; Cunningham et al., 2004). GALP mRNA expression differs slightly from galanin with GALP confined to the hypothalamic arcuate nucleus, median eminence (Jureus et al., 2000; Larm and Gundlach, 2000), and posterior pituitary (Jureus et al., 2000; Shen et al., 2001). In male rats, central injections of GALP have been shown to increase luteinizing hormone (LH) release, activate fos expression throughout the diencephalons and midbrain; however, a large density of fos expression is observed within the medial preoptic area (mPOA, Fraley et al., 2003; Lawrence et al., 2003b). Central administration of GALP is known to stimulate GnRH-mediated LH secretion, food intake, and male-typical sexual behaviors—many of these actions appear to be mediated via the mPOA (Matsumoto et al., 2001; Fraley et al., 2003, 2004; Patterson et al., 2006a). Furthermore, we have reported that in castrated males, intracerebral injections of GALP stimulated sexual behaviors; however, GALP was not able to stimulate the ejaculatory response in these animals (Fraley et al., 2004). In diabetic male rats where GALP expression, plasma LH, and sexual behavior are virtually nonexistent, we found that central administration of GALP restored both LH in male rats and normalized mounts and intromissions; however, GALP was again not able to restore ejaculatory behaviors (Stoyanovitch et al., 2005b). This characteristic to restore mounts and intromissions, but not ejaculatory behaviors in male rats is reminiscent of dopaminergic effects in the mPOA reported by others (Hull et al., 1986, 1995; Bitran and Hull, 1987; Bitran et al., 1988).

Dopaminergic neurons within the anteroventral-posteroventral nucleus (AVPV) project to the areas of the hypothalamus known to influence male-typical sexual behaviors (for review see,

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Liu et al., 1997). Similar to GALP, dopamine has been shown to restore mounts and intromissions, but not ejaculations in castrated male rats (Hull et al., 1995). It is unknown whether GALP directly stimulates the SDN-POA, thus increasing sex behavior, or if GALP stimulates dopamine release within the SDN-POA that in turn stimulate male sex behavior.

The purpose of these studies was to determine if centrally administered GALP could maintain its stimulatory effects on male sex behavior and LH secretion in the absence of dopaminergic input to the mPOA. To accomplish this goal, we selectively eliminated dopaminergic neurons within the mPOA, and attempted to restore sex behavior with centrally administered injections of GALP.

2. Materials and methods

2.1. Animals

Adult male Long-Evans rats (280–300 g) were purchased from Harlan (Indianapolis, IN, USA) and housed in individual cages in an American Association for the Accreditation of Laboratory Animal Committees (AAALAC)-approved vivarium. Commercial rodent chow and water were given *ad libitum* and animals were maintained on a 12L:12D cycle with lights on at 07:00 h. All animal procedures were approved by the Hope College Animal Care and Use Committee (HCACUC).

2.2. Cannulation

All rats were anesthetized with a subcutaneous injection of a ketamine (100 mg/mL ketamine; 20 mg/mL xylazine; 10 mg/mL acepromazine) cocktail and placed in a stereotaxic instrument (MyNeuroLab, Benchmark Angle One™ Stereotaxic Instrument). A single incision was made on the midline of scalp to visualize bregma. The instrument was zeroed (centered ear bars) and a stainless steel 26-gauge cannula (Plastics One, Roanoke, VA) was inserted into the mPOA (0.4 mm lateral to the midline, 0.5 mm posterior to bregma, and 8.4 mm inferior to dura matter). The cannula was fixed to the skull with two support screws and covered with Cranioplastic cement (Plastics One, Roanoke, VA). A 31-gauge dummy cannula was inserted into the guide cannula when not in use to protect injection site from debris. Animals were housed individually in their home cage and allowed 7 days to recover, during which time they were handled daily. At the end of study, animals were euthanized with an overdose of pentobarbital (400 mg/kg body weight, Fatal Plus; Vortech, Dearborn, MI), brains were removed and frozen at −80 °C until further analysis.

2.3. Microinjections: DATSAP and SAP

Rats were anesthetized with a subcutaneous injection of a ketamine (100 mg/mL ketamine; 20 mg/mL xylazine; 10 mg/mL acepromazine; 5.0:2.5:1.0 ratio, respectively). After a surgical plane of anesthesia was achieved, either a monoclonal antibody against the dopamine transporter DATSAP (DATSAP, Chemicon International, Inc., Temecula, CA, USA) (in phosphate buffer, pH 7.4) or control, unconjugated saporin (SAP, Advanced Targeting Systems, San Diego, CA, USA) was injected into the mPOA. The saporin molecule is a ribosomal inhibitor that is incapable of trans-membrane transport. Thus, the saporin conjugated to the monoclonal antibody against the dopamine transporter (DAT) allows the saporin access to the cytoplasm when the DAT molecule is internalized. Once access has been gained, the saporin molecule is capable of killing the cell—thus this is an excellent method of specifically lesioning DA inputs to a specific target site. For an overview of this technique (see, Ritter et al., 2001, 2003; Fraley et al., 2002; l'Anson et al., 2003; Rinaman, 2003; Wiley et al., 2003; Fraley, 2006). Microinjections were given through a 30-gauge stainless steel needle attached to polyethylene tubing and a 1.0 μL Hamilton Syringe (Hamilton Inc., Reno, NV). A total of 100 nL (42 ng) of DATSAP or SAP (n = 16 per injection type) was injected bilaterally into the mPOA over a 6–7 min time period.

2.4. Experiment 1: confirmation of DATSAP lesions of the mPOA

Two weeks following the DATSAP or SAP control solution, males were placed into a testing arena (polyethylene housing cage) with a steroid-primed female (10 μL estradiol benzoate in 0.1 mL safflower oil injected s.c. 48 h prior to test and 500 mg progesterone in 0.1 mL safflower oil injected s.c. 4 h prior to test) for 30 min. All rats were sexually experienced and had been previously habituated to the testing chamber following surgical procedures. All tests were performed during the early photophase (08:00–12:00 h). We have published this time frame for GALP's maximal effectiveness to stimulate male sexual behavior (Fraley et al., 2004; Stoyanovitch et al., 2005a). All sexual behavior tests were videotaped and analyzed by individuals unaware of treatment groups. Analyses of behaviors were completed as described previously by our laboratory (Stoyanovitch et al., 2005b). During the 30 min test period mounts, intromissions, and ejaculations as well as latency to these behaviors were recorded. Mounts were recorded if the male approached the female from the rear and placed both forelegs firmly on her flanks and locked his hips. Intromissions were recorded if the foregoing behavior was noted with the addition of a clear pelvic thrust followed by genital grooming. An ejaculation was recorded if an intromission was observed followed directly with pelvic thrusting, genital grooming, and a period of malaise.

2.5. Experiment 2: GALP-induced sex behavior

All DATSAP-lesioned and SAP control rats received ICV injection of 3.0 μL GALP (5.0 nmol in 0.1 M phosphate buffer (PB), pH 7.4—the dose established to stimulate male sexual behavior Fraley et al., 2004), and placed in testing arena with a steroid-primed, sexually receptive female (10 μg estradiol benzoate in 0.1 mL safflower oil injected s.c. 48 h prior to test and 500 mg progesterone in 0.1 mL safflower oil injected s.c. 4 h prior to test). All rats were sexually experienced and had been previously habituated to testing chamber following surgical procedures. Males were placed with the females for 30 min per trial. All tests were performed during the early photophase (08:00–12:00 h) and analyzed as described above.

2.6. Experiment 3: ICV GALP effects on food intake

As an internal control for the ICV GALP effects, we measured food intake and body weight following ICV GALP or vehicle in both DATSAP and SAP controls. Galanin-like peptide is known to have a bimodal effect on food intake initially having an orexigenic effect (Matsumoto et al., 2002) followed by a 24 h anorexigenic effect and loss of body weight (Lawrence et al., 2002, 2003a). Recent evidence suggests that the initial orexigenic effect of GALP is mediated by the mPOA (Patterson et al., 2006b). Thus we measured food intake for 2 h after ICV GALP or vehicle in both DATSAP and SAP control rats. We further measured food intake and body weight at 24 h after ICV injections.

2.7. Experiment 4: ICC for fos and tyrosine hydroxylase and effects on LH secretion

All male rats received an ICV injection of either 3.0 μL GALP or vehicle. After 2 h, the animals were deeply anesthetized with Fatal Plus (intraperitoneal 400 mg/kg body weight, Fatal Plus; Vortech, Dearborn, MI) and the thoracic cavity was exposed. Blood was immediately removed via cardiac puncture and plasma collected and stored at −20 °C for LH analyses via radioimmunoassay (RIA). The animals were then perfused with heparinized saline (50 mL 0.9% NaCl, 1 IU/100 mL Na heparin) followed by aldehyde fixative (4% paraformaldehyde in 0.1 M PB, pH 7.4). Brains were removed, and placed in the same fixative for 5 h at 4 °C then put into 20% sucrose in PB overnight, then frozen and stored at −80 °C until sectioned.

2.7.1. Tissue preparation and immunocytochemistry

Four parallel series of 40 μm coronal sections of brain tissue were cut on a sliding microtome (American Optical Company, Buffalo, NY, USA) from the diagonal band of Broca (DBB) through the mammillary bodies, and stored in cryopreservative (0.9% NaCl, 30% sucrose, 1% polyvinylpyrolidine mw 40,000, 30% ethylene glycol in 0.05 M PB) solution at −20 °C until processed.

Immunocytochemistry for fos was performed on one set of hypothalamic sections by a standard ABC (avidin/biotin complex) reaction, as previously described (Fraley et al., 2003). Briefly, sections were washed in PB and pre-incubated in blocking solution (PB, 1.0% non-fat dried milk (NFM), 0.05% Triton X-100) for 1 h at room temperature (RT). Sections were then transferred to blocking solution containing rabbit anti-fos polyclonal antibody (1:50,000, Oncogene Research Products, La Jolla, CA, USA) and incubated for 48 h at 4 °C with agitation. After three PB washes, sections were incubated for 3 h at RT in blocking solution with secondary antibody (1:500, biotinylated-anti-rabbit, Vector Laboratories, Burlingame, CA, USA). Fos immunoreactivity was visualized with Ni-3,3′5,5′ diaminobenzidine (DAB) as the chromagen to produce a blue-black reaction product (DAB Chromagen Kit No. PK6100, Vector Laboratories). Sections were mounted on Superfrost Plus slides (VWR Scientific, West Chester, PA, USA), air-dried, dehydrated in graded ethanol, and cleared with Citrosolv, after which coverslips were applied.

Staining for tyrosine hydroxylase (TH)-ir was done as described above for fos, except that the primary antibody was a mouse anti-TH monoclonal used at 1:2000 dilution (Vector Labs, Indianapolis, IN).

2.7.2. Analyses of immunocytochemistry

Fos-positive cells were examined as darkly stained-nuclei. Sections were examined qualitatively throughout the mPOA and remaining hypothalamic sections for GALP-induced fos expression as reported previously (Fraley et al., 2003). Tissues were analyzed qualitatively for the presence or absence of fos-ir and TH-ir.

2.7.3. Radioimmunoassays

Plasma LH concentrations were measured via RIA at Northwestern University (Evanston, IL, USA). The LH assay was run with reagents from the NIH and the