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Central neuropeptide FF reduces feed consumption and affects hypothalamic chemistry in chicks

Mark A. Cline *, Wint Nandar, J. Orion Rogers

Department of Biology, Radford University, P.O. Box 6931, Radford, VA 24142, USA

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Abstract

Information on the physiological functions of neuropeptide FF; NPFF, a morphine modulating octapeptide in avians is lacking. Thus, we designed a study to investigate the effects of central NPFF with particular emphasis on appetite-related processes. Cobb-500 chicks were intracerebroventricularly (ICV) injected with 0, 4.16, 8.32 or 16.6 nmol NPFF, and feed and water intake were quantified. Feed intake was linearly decreased as NPFF dose increased, and this effect decayed over time and was not significant by 120 min post-injection. Water intake was not affected by ICV NPFF. In a second exp, we observed that naloxone completely reversed the NPFF-induced decrease in feed intake. The amount of time a visible marker took to travel through the total length of the alimentary canal linearly increased as NPFF dose increased. We measured neuronal activation in the lateral hypothalamus (LH), paraventricular nucleus (PVN) dorsomedial nucleus (DMN) and ventromedial hypothalamus (VMN) of the hypothalamus, and nucleus dorsomedialis posterior thalami (DMP) of the thalamus. The DMN, DMP, PVN and VMH were all activated by ICV NPFF while the LH was not affected. Finally, we determined that the anorexigenic effect of ICV NPFF is primarily behavior specific, since behaviors unrelated to ingestion were not increased the same duration of time as was consumatory pecking. We conclude that NPFF causes anorexigenic effects in chicks that are primarily behavior specific.

Keywords: Appetite; Behavior; Chick; F-8-F-amide; Hypothalamus; Feed intake; Neuropeptide FF; NPFF; Satiety

1. Introduction

The FMRFamide-like octapeptide, neuropeptide FF (NPFF), also known as F-8-F-amide, or morphine modulating neuropeptide, was first identified in the bovine brain as a morphine modulator (Yang et al., 1985). As an endogenous anti-opioid peptide (Rothman, 1992; Cesselin, 1995) NPFF has specifically been isolated from the hypothalamus, posterior pituitary and medulla of rodents (Kivipelto and Panula, 1991; Aarnisalo and Panula, 1995; Panula et al., 1996). In addition to reversing opioid induced analgesia (Roumy and Zajac, 1998), NPFF also mediates other effects (see Panula et al., 1996) including pressor and tachycardiac responses (Allard et al., 1995; Roth et al., 1987) and effects on the gut (Schmidt et al., 1989; Fehmann et al., 1990). In rats NPFF affects feed (Murase et al., 1996; Nicklous and Simansky, 2003) and water (Sunter et al., 2001) intake. Interestingly, NPFF shares receptor sequence identity with NPY (Mollereau et al., 2001), the most potent orexigenic of all peptides.

NPFF binds to two G-protein coupled receptors, NPFF1 and NPFF2 in mammals (Bonini et al., 2000; Kotani et al., 2001) that cause activation of $G_{i/o}$ (Hinuma et al., 2000; Kotani et al., 2001). NPFF2 appears to exert most of NPFF's effects (Ankö and Panula, 2006), and some have suggested that NPFF1 is the prolactin-releasing peptide receptor (Chartrel

* Corresponding author. Tel.: +1 540 831 6431; fax: +1 540 831 5129.
E-mail address: mcline@radford.edu (M.A. Cline).

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et al., 2006). The C-terminal-RFamide is essential for NPFF receptor activation (Mazarguil et al., 2001). NPFF modulates endogenous opioid function by affecting neuronal Ca^{2+} concentration (Roumy and Zajac, 1998). However, NPFF does not bind to opioid receptors (Allard et al., 1989).

The physiological function of NPFF in avians is poorly understood. Thus, in the present work, we have investigated the effects of intracerebroventricular (ICV) NPFF on feed and water intake using Cobb-500 chicks. The role of opioid receptors in the NPFF anorexigenic signal was also studied. We subsequently determined which hypothalamic nuclei are associated with the anorexigenic effects of NPFF by measuring neuronal activation in the lateral hypothalamus (LH), ventromedial (VMN) hypothalamus, paraventricular nucleus (PVN) and dorsomedial nucleus (DMN) of the hypothalamus in addition to the nucleus dorsomedialis posterior thalami (DMP) of the thalamus. Additionally, we studied the influence of NPFF on alimentary canal transit rate and on behaviors not related to ingestion as an indicator if NPFF-induced satiety is partly due to other physiological modifications and non-specific effects.

2. Experimental procedures

2.1. Animals

Morning of hatch Cobb-500 broiler chicks from breeders 30 to 40 weeks of age were obtained from a commercial hatchery. They were caged individually in a room at 30 ± 2 °C and 50 ± 5% relative humidity with ad libitum access to a mash diet (20% crude protein) and tap water. All trials were conducted 4 d post-hatch. The sequential experiments reported consisted of 4 hatches (2 for Exp 1, 1 for Exp 2 and 3 and 1 for Exp 4 and 5). All experimental procedures were performed according to the National Research Council publication, Guide for Care and Use of Laboratory Animals and were approved by the Radford University Institutional Animal Care and Use committee.

2.2. Intracerebroventricular (ICV) injection procedure

Chicks were injected using a method adapted from Davis et al. (1979). The head of the chick was briefly inserted into a restraining device that left the cranium exposed and allowed for free-hand injection. Injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 2 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. Injection depth was controlled by placing a plastic tubing sheath over the needle. The needle remained at injection depth for 10 s post-injection to reduce backflow. Chicks were assigned to treatments at random. NPFF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2; American Peptide Co., Sunnyvale, CA, USA) was dissolved in artificial cerebrospinal fluid (aCSF) for a total injection volume of 5 μL with 0.1% Evans Blue dye to facilitate injection site localization. After data collection, the chick was decapitated and its head sectioned along the frontal plane to determine site of injection. Any chick without dye present in the lateral ventricle system was eliminated from analysis. Numbers of chicks in an exp are provided in Section 3.

2.3. Exp 1: effect on feed and water intake

Chicks, fasted for 180 min, were randomly assigned to receive 0, 4.16 nmol (4.5 μg), 8.32 nmol (9.0 μg) or 16.6 nmol (18.0 μg) NPFF by ICV injection. After injection chicks were returned to their individual cages and given ad libitum access to both feed and water. Both feed and water intake were concurrently measured (0.01 g) every 30 min for 180 min post-injection. Data were analyzed using analysis of variance at each time point. The model included NPFF dose, replicate and the interaction of NPFF dose with replicate. If significant NPFF dose effects were found, a Duncan Multiple Range test was used to separate the means. NPFF dose effects were partitioned into linear and quadratic contrasts to determine dose relationships at each time period. Water weight (g) was converted to volume (ml; 1 g = 1 ml). Statistical significance was set at $P < 0.05$ for all experiments.

2.4. Exp 2: opioid receptor antagonism

The experimental procedures were identical to those in Exp 1 except that chicks were randomly assigned to receive 0, 8.32 nmol NPFF, 38.3 nmol naloxone (Sigma–Aldrich Co., St. Louis, MO, USA) and 8.32 nmol NPFF + 38.3 nmol naloxone. The dose of naloxone was based on Savory et al. (1989), and the dose of NPFF was based on the results of Exp 1. If significant treatment effects were found, Tukey's method of multiple comparison was used to separate the means. A single replicate was conducted, thus replicate was removed from the model.

2.5. Exp 3: total alimentary canal transit rate

Non-fasted chicks received the same treatments as in Exp 1. Immediately following injection, chicks were gavaged into the crop with a feed slurry at a mass of 4.0% body weight into the crop. The feed slurry was made by mixing 45% chicken feed ground to a fine powder with 54% tap water and 1% ferric oxide on a weight basis. Chicks that vomited after gavage were removed