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Effect of nociceptin/orphanin FQ on feeding behavior and hypothalamic neuropeptide expression in layer-type chicks

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ABSTRACT

Nociceptin/orphanin FQ (N/OFQ) was identified in 1995 as the endogenous ligand for the orphan G_i/G_o-coupled opioid receptor-like 1 receptor (NOP_1). Exogenous N/OFQ increases food intake in mammals, but its effect and mode of action in chicks are not fully known. We report herein that N/OFQ (5.0 nmol) has a stimulatory effect on food intake in layer-type chicks over a 2-h period after intracerebroventricular (icv) injection. Thirty minutes after central injection of N/OFQ (5.0 nmol) the concentration of agouti-related protein (AGRP) mRNA in the diencephalon increased, while cocaine- and amphetamine-regulated transcript (CART) mRNA decreased. However, concentrations of neuropeptide Y, proopiomelanocortin and glutamate decarboxylase mRNAs, and of catecholamines and excitatory amino acids were not affected. Simultaneous administration of α-melanocyte stimulating hormone (α-MSH: 1.0 pmol), a competitor of AGRP, completely blocked the orexigenic effect of N/OFQ (5.0 nmol). These data suggest that N/OFQ functions in layer chicks as an orexigenic peptide in the central nervous system, and that the AGRP and the CART neurons may mediate this function, as in mammals.

1. Introduction

Nociceptin/orphanin FQ (N/OFQ) is an opioid-related heptadecapeptide identified as the endogenous ligand for the orphan G_i/G_o-coupled opioid receptor-like 1 receptor (NOP_1) (Meunier et al., 1995; Reinscheid et al., 1995). The peptide exhibits structural homology with dynorphin A (over 60%; Meunier et al., 1995), but does not have any pharmacological actions at μ-, δ- and κ-opioid receptors (Reinscheid et al., 1998). N/OFQ and NOP_1 are widely distributed in the brain (Mollereau and Mouledous, 2000; Reinscheid et al., 2000), consistent with involvement in the control of a variety of biological functions: pain (Darland and Grandy, 1998), anxiety and stress (Jenck et al., 1997), reward and dependence (Di Giannuario and Pieretti, 2000), memory and learning (Sandin et al., 1997; Hiramatsu and Inoue, 1999), and sexual behavior (Sinchak et al., 1997).

N/OFQ has also been shown to stimulate feeding behavior in rats following intracerebroventricular (icv) administration (Pomonis et al., 1996; Polidori et al., 2000; Economidou et al., 2006). This effect has also been induced by injecting N/OFQ into specific brain sites, namely, the nucleus accumbens (NAC), ventromedial hypothalamus, paraventricular nucleus, arcuate nucleus (ARC), and the nucleus of the solitary tract (Stratford et al., 1997; Polidori et al., 2000). The ARC, in particular has been shown to be the most sensitive site of action for N/OFQ in rats (Polidori et al., 2000), and contains peptidergic cell types, containing neuropeptide Y (NPY), agouti-related protein (AGRP), proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), that are strongly implicated in the stimulatory and inhibitory controls of food intake (for review see Kalra et al., 1999). The mechanisms underlying the orexigenic effects of N/OFQ have been investigated in the rat. Bewick et al. (2005) showed that N/OFQ treatment stimulates an increase in AGRP, and a decrease in CART release. It is known that AGRP is an orexigenic peptide that competes with α-melanocyte stimulating hormone (α-MSH) for melanocortin receptors, whereas CART exerts an inhibitory effect on food intake (Kalra et al., 1999).

As for other effects of N/OFQ in the central nervous system (CNS), centrally administered N/OFQ decreases extracellular dopamine (DA) levels in the NAC and striatum (Murphy et al., 1996; Murphy and Maidment, 1999; Marti et al., 2004), and increases GABA and glutamate (Glu) overflow in the ventral tegmental area (Murphy and Maidment, 1999). In the CNS, virtually all GABA is produced by decarboxylation of L-glutamic acid by two enzymes (GAD1 and GAD2): GAD1 regulation is slow, whereas the regulation of GAD2 is rapid, reflecting its role in the synthesis of GABA for synaptic release and the requirement be able to respond

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quickly to sudden increases in demand for GABA (McCarthy et al., 1994; Mitsushima et al., 1994). Schlicker and Morari (2000) summarized data from mammals showing that N/OFQ stimulates Glu, GABA and monoamines (NA and DA) release from brain slices and synaptosomes. Taken together, these findings suggest that the orexigenic effects of N/OFQ in the CNS are associated with changes in the hypothalamic AGRP, CART, catecholamines and excitatory and inhibitory amino acids.

In birds, most neuropeptides involved in appetite control have effects similar to those observed in mammals, but some have an opposite effect (for review see Furuse et al., 2007). As in mammals, icv injection of N/OFQ increase food intake in meat-type (broiler) chickens (Abbasnejad et al., 2005), and this effect is blocked by the injection of bicuculline, a $GABA_A$ receptor antagonist (Tajalli et al., 2006). However, relatively little is known about the central mechanisms through which N/OFQ exerts its orexigenic action in birds.

The aim of this present series of experiments was to establish in layer-type chicks, whether (1) icv injection of N/OFQ increases food consumption under *ad libitum* feeding conditions, (2) icv N/OFQ at a dose that stimulates feeding behavior affects the expression of NPY, AGRP, POMC, CART and glutamate decarboxylase (GAD) genes, the concentrations of L-DOPA, DA, noradrenaline (NA), adrenaline (Ad), and the levels of Glu and aspartate (Asp) in the diencephalon, and (3) icv co-injection of N/OFQ and α-MSH decreases the orexigenic effect of N/OFQ.

2. Materials and methods

2.1. Animals

Male White Leghorn chicks (Julia light; Akita Co. Ltd., Hiroshima, Japan) were caged individually in a room with 24-h lighting and at a temperature of 30 °C. They were given free access to a commercial starter diet (Nichiwa Sangyo Co. Ltd., Kobe, Japan) and water. They were allocated to experimental groups based on body weight so that the average body weight was as uniform as possible for each treatment. The handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

2.2. Experimental procedures

Chicks (3–5 day-old) were given icv injections (10 μl) of saline, or N/OFQ (0.2, 1.0 or 5.0 nmol) and food intake was measured after 30, 60 and 120 min. A further group was similarly injected with saline, N/OFQ (5.0 nmol), α-MSH (1.0 pmol) or N/OFQ (5.0 nmol) together with α-MSH (1.0 pmol) and food intake was also measured after 30, 60 and 120 min. Food intake was calculated after drug injection by measuring the weight of feeders using an electronic digital balance with a precision of ±1 mg. Further groups of chicks were injected with icv N/OFQ (5.0 nmol) and sacrificed at 15 and 30 min to measure diencephalic neuropeptide mRNAs, excitatory amino acids or catecholamines. All drugs were purchased from Sigma (St. Louis, MO, USA). The drugs were administered according to Davis et al. (1979) using a microsyringe dissolved in 0.85% saline containing 0.1% Evans Blue. The control groups were injected with saline containing Evans Blue. Each chick was injected once only with saline or drug(s) between 12:00 and 2:00 p.m. At the end of the experiments the chicks were sacrificed by decapitation and the location of each injection site was confirmed by the presence of Evans Blue dye in the lateral ventricle. If Evans Blue was not present, the tissue donor chick was not included in the experimental results. For studies requiring measurements of diencephalic neuropeptide mRNAs, excitatory amino acids or catecholamines, the diencephalon was dissected according to a chick brain atlas (Kuenzel and Masson, 1988). The tissue dissected included hypothalamic areas involved in the regulation of feeding behavior, namely the lateral hypothalamic area and the ventromedial, paraventricular (PVN) and arcuate hypothalamic nuclei (Kuenzel et al., 1999). The samples were weighed, frozen on dry ice and stored at −80 °C prior to analyses.

2.3. Measurement of neuropeptide mRNAs, excitatory amino acids and catecholamines

2.3.1. Measurement of POMC, NPY, CART, AGRP, GAD1 and GAD2 mRNAs

RNA was isolated using Trizol (Invitrogen, CA, USA) according to the manufacturer's instructions. To rule out the possibility that PCR products resulted from the amplification of contaminating genomic DNA, RNA samples were treated with DNase I using a DNA-free kit (Ambion, Austin, USA). Total RNA (480 ng) was reverse transcribed at 42 °C for 15 min in 10 μl of 1× PrimeScript buffer containing 50 μM random primers and Prime Script RT Enzyme Mix I (Takara, Tokyo, Japan). The reaction product was subjected to Q-PCR using the Light Cycler system (Roche Applied Science, IN, USA) following the manufacturer's instructions. Briefly, following a denaturation step at 95 °C for 10 s, PCR was carried out using a protocol of 95 °C for 5 s and 60 °C for 20 s in a 20 μl buffer containing 1× SYBR Premix EX Taq (Takara, Tokyo, Japan) and 0.2 μM of each primer. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The primers used were as follows: POMC, 5′-aacagcaagtgccaggacc-3′ (sense) and 5′-atcacgt acttgcggatgct-3′ (antisense); AGRP, 5′-aggccagacttggatcagatg-3′ (sense) and 5′-actccaggaggcggacac-3′ (antisense); NPY, 5′-ggcactac atcaacctcatc-3′ (sense) and 5′-ctgtgctttccctcaacaa-3′ (antisense); CART, 5′-ccgcactacgagaagaag-3′ (sense) and 5′-aggcacttgagaaga aagg-3′ (antisense); GAD1, 5′-ttcagtcacttggaatccacac-3′ (sense) and 5′-gattgcagccttgaagtattcc-3′ (antisense); GAD2, 5′-agaggggaa aggcttcttatgg-3′ (sense) and 5′-ctcaaacaacctccaacactgc-3′ (antisense) and GAPDH, 5′-gccgtcctctctggcaaa-3′ (sense) and 5′-tgtaaacc atgtagttcagatcgatga-3′ (antisense). To normalize the data, the ΔC_T was calculated for each sample by subtracting the average C_T of GAPDH from the average C_T of the gene of interest. For relative quantification, the ΔC_T was averaged for the defined control group and was then subtracted from the ΔC_T of each experimental sample to generate the $\Delta\Delta C_T$. The $\Delta\Delta C_T$ was then used to calculate the approximate fold difference, $2^{-\Delta\Delta C_T}$. The results were expressed as the gene of interest mRNA/GAPDH mRNA ratio. Each PCR run included a no template control and replicates of control and unknown samples.

2.3.2. Measurement of excitatory amino acids

Tissue samples were homogenized in 10 volumes of 2.0% w/v sulfosalicylic acid solution. The homogenate was centrifuged at 10,000 rpm for 60 min and the supernatants were filtered through a 0.22 μm filter. Then, 50 μl of the filtrate was analyzed for Glu and Asp in an amino acid analyzer (Aminolyzer 21, SIC, Tokyo, Japan).

2.3.3. Measurement of concentrations of catecholamines

Tissue was homogenized in 10 volumes of 0.1 N $HClO_4$. The homogenates were centrifuged at 13,000 rpm for 15 min, and supernatants were filtered through a 0.22 μm filter before injection (100 μl) into a high performance liquid chromatography system (Tosoh, Tokyo, Japan) with a 150 × 2.1 mm ODS column (CA-5ODS, EICOM, Kyoto, Japan) for the measurement of monoamines and their metabolites. Column temperature was kept at 40 °C by a thermocontroller (TSK CO-8000; Tosoh, Tokyo, Japan). The solvent delivery system (TSK CCPD; Tosoh, Tokyo, Japan) contained 2.5 mM of 1-octanesulfonic acid sodium salt (SOS), 20 μM Na_2ED-