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General and Comparative Endocrinology 144 (2005) 264–269
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Comparison of mammalian prolactin-releasing peptide and Carassius RFamide for feeding behavior and prolactin secretion in chicks

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Received 30 January 2005; revised 17 June 2005; accepted 23 June 2005
Available online 22 August 2005

Abstract

Prolactin-releasing peptide (PrRP) was named for its originally reported effects as a prolactin (PRL) secretagogue in mammals. Carassius RFamide (C-RFa) is an orthologous PRL secretagogue in fishes and a gene encoding a 20-amino acid peptide of identical sequence is present in the chicken. These facts suggest that C-RFa is a putative chicken PrRP. However, no information is available for the physiological effects of C-RFa in chickens. Therefore, in the present study, we compared the effect of intracerebroventricular (ICV) injection of C-RFa and mammalian PrRP (mPrRP) on feeding behavior and plasma PRL, growth hormone (GH), and corticosterone (CORT) concentrations. ICV injection of C-RFa did not affect feeding behavior of chicks while mPrRP was stimulatory. The injection of C-RFa also did not significantly affect plasma PRL, GH, and CORT concentrations. In contrast, ICV injection of mPrRP exerted similar effects to those reported in mammals by increasing plasma CORT and decreasing GH concentrations. Additionally, the peptide induced an unexpected inhibitory effect on plasma PRL concentrations. Overall, these data suggest that an as yet unidentified peptide that shares some functional similarities with mPrRP is present in birds, but that the physiological role of the avian 20-amino acid C-RFa peptide remains to be determined.

Keywords: Carassius RFamide; Chick; Corticosterone; Feeding; Growth hormone; Intracerebroventricular injection; Prolactin; Prolactin-releasing peptide

1. Introduction

Prolactin-releasing peptide (PrRP, Fig. 1) was identified as a ligand for an orphan receptor and was originally proposed to be a stimulator of prolactin (PRL) release in mammals (Hinuma et al., 1998; Matsumoto et al., 1999). Physiological effects of the peptide on the neuroendocrine system are broader, including inhibition of growth hormone (GH) release (Iijima et al., 2001) and stimulation of luteinizing hormone, follicle-stimulating hormone, oxytocin, and adrenocorticotrophic hormone release (Maruyama et al., 1999a,b; Matsumoto et al., 2000; Seal et al., 2000). Central administration of PrRP was also reported to decrease food intake in rats,

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doi:10.1016/j.ygcen.2005.06.012

C-RFa	**SPEIDPFWYVGRGVRPIGRF**-NH_2
Rat PrRP31	SRAHQHSMETRT**PDINPAWYTGRGIRPVGRF**-NH_2
Bovine PrRP31	SRAHQHSMEIRT**PDINPAWYAGRGIRPVGRF**-NH_2
Human PrRP31	SRTHRHSMEIRT**PDINPAWYASRGIRPVGRF**-NH_2

Fig. 1. Amino acid sequence of C-RFa and mPrRPs. These amino acid sequences were reported by Fujimoto et al. (1998) and Hinuma et al. (1998), respectively. The sequence of C-RFa is identical in teleost fish and the chicken.

suggesting that PrRP is involved in the regulation of behavior in mammals, in addition to its neuroendocrine effects (Lawrence et al., 2000).

We recently reported that intracerebroventricular (ICV) injection of mammalian PrRP (mPrRP) stimulated feeding behavior in chicks in contrast to the inhibitory effect observed in mammals (Tachibana et al., 2004). This suggested the existence of an endogenous chicken PrRP that is involved in the regulation of food intake. One possible candidate is Carassius RFamide (C-RFa, Fig. 1), a PRL secretagogue in teleost fish that is thought to be a piscine orthologue of mPrRP (Fujimoto et al., 1998; Moriyama et al., 2002; Satake et al., 1999; Seale et al., 2002). A C-RFa-like nucleotide sequence is present in the chickEST database (http://chick.umist.ac.uk/) and the translated 20-amino acid peptide sequence is identical to that of C-RFa. This suggests that C-RFa may be a chicken PrRP. However, no reports are available on the effect of C-RFa on feeding behavior and neuroendocrine secretion in chicks.

The purpose of the present study was therefore to investigate whether central administration of C-RFa influences food intake and plasma PRL, GH, and corticosterone (CORT) concentrations. Furthermore, the effects of C-RFa were compared with those of mPrRP.

2. Methods

2.1. Animals

Day-old male layer-type chicks (purchased from Murata Hatchery, Fukuoka, Japan) were maintained in a room at 30 °C under continuous lighting. The birds were freely given a commercial diet (Toyohashi Feeds and Mill, Aichi, Japan) and water except elsewhere noted. All experimental procedures were done according to the guidance for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No.105) and Notification (No.6) of the Government.

2.2. ICV injection

Synthetic C-RFa (as reported by Seale et al., 2002) and rat PrRP-31 (purchased from Peptide Institute, Osaka, Japan) were dissolved in a 0.1% Evans Blue solution prepared in a saline solution and the injected volume was 10 μl. The control group was injected with the same volume of this Evans Blue solution. ICV injections were performed according to the method previously reported (Davis et al., 1979). Briefly, the head of the chick was inserted in an acrylic device, which was opened a hole at the top plate. When the head was held, the hole will situated immediately over the left lateral ventricle. After that, a microsyringe was inserted into the left lateral ventricle through the hole and the drug was injected. Chicks could move and eat immediately after the injection. This method is not stressful for chicks since the ICV injection of saline solution, which was used as the control group in the present study, did not affect feeding behavior (Furuse et al., 1999) and corticosterone release (Saito et al., 2005) when compared with the intact chick. Therefore, we did not anesthetize chicks for the injection.

After each experiment was finished, chicks were sacrificed with an intraperitoneal overdose of sodium pentobarbital and their brains were then removed. Confirmation of successful intraventricular injection was made by observation of the presence of Evans Blue dye in the lateral ventricle. The results obtained from chicks that did not have the dye in the ventricle were not used.

2.3. Experiment 1: effects of ICV injection of C-RFa and mPrRP on food intake

The effects of ICV injection of C-RFa and PrRP on food intake of chicks were examined. Each chick (3 days old) was injected with saline (control), 94 pmol C-RFa, 375 pmol C-RFa, 94 pmol mPrRP or 375 pmol mPrRP under ad libitum feeding conditions. The doses were determined according to our previous study (Tachibana et al., 2004). Food intake was determined at 2 h after the injection. The number of chicks in each group was as follows: saline, 7; 94 pmol C-RFa, 5; 375 pmol C-RFa, 6; 94 pmol mPrRP, 5; 375 pmol mPrRP, 6.

2.4. Experiment 2: effects of ICV injection of C-RFa and mPrRP on plasma PRL, GH, and CORT concentrations

For the C-RFa study, chicks (3 days old) received ICV injections of 0 (control), 94 or 375 pmol C-RFa under ad libitum feeding conditions. After the injection, the diet and water were removed from their home cages. The blood was collected by heart puncture at 30 min after the injection. Plasma PRL and GH concentrations were measured by radioimmunoassay. Briefly, chicken PRL and GH (provided by Dr. A.F. Parlow, Pituitary Hormones and Antisera Center, National Hormone and Peptide Program, Harbor-University of California Los Angeles Medical Center, Torrance, CA) were labeled with ^{125}I by the chloramine-T method. The labeled PRL and GH were purified by gel filtration using NAP-10