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Review

Avian and murine neurosecretory protein GL participates in the regulation of feeding and energy metabolism

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ABSTRACT

Probing previously unknown neuropeptides and/or peptide hormones is essential for our understanding of the regulation of energy homeostasis in the brain. We recently performed a cDNA subtractive screening of the chicken hypothalamus, which contained one of the feeding and energy metabolic centers. We found a gene encoding a novel protein of 182 amino acid residues, including one putative small secretory protein of 80 amino acid residues. The C-terminal amino acids of the small protein were Gly-Leu-NH_2, and as a result, the small protein was termed neurosecretory protein GL (NPGL). Subcutaneous and intracerebroventricular infusions of NPGL increased body mass gain in chicks, suggesting a central role for this protein in regulating growth and energy homeostasis. A database search revealed that the *Npgl* gene is conserved in vertebrates, including mice and rats. This review summarizes the advances in the characterization, localization, and biological action of NPGL, in birds and rodents.

1. Introduction

Neuropeptides, and/or peptide hormones, are bioactive peptides synthesized in the central and peripheral nerves, and in various tissues. They act as transmitters, modulators, and/or secretory hormones (Marder, 2012). In the brain, they contribute to various physiological processes, such as sensory, autonomic, neuroendocrine, behavioral, emotional, and cognitive processes (Hökfelt et al., 2000). The identification of previously unknown bioactive peptides can help reveal the molecular mechanisms that underlie brain functions. Prior to the last decade, several neuropeptides were discovered in the mammalian brain: neuromedin S (NMS), TLQP-21, nesfatin-1, and neuroendocrine regulatory peptide (NERP) (Mori et al., 2005; Bartolomucci et al., 2006; Oh-I et al., 2006; Yamaguchi et al., 2007). Previous studies have demonstrated that these neuropeptides are involved in feeding behavior (Ida et al., 2005; Bartolomucci et al., 2006; Oh-I et al., 2006; Toshinai et al., 2010). However, there have been few reports describing the discovery of new bioactive peptides in vertebrates over the last decade. It was recently reported that nonadecaneuropeptide derived from Acyl-CoA binding domain-containing 7 is a novel anorexigenic factor in mice (Lanfray et al., 2016).

We have investigated LPXRFamide peptides, including gonadotropin-inhibitory hormone (GnIH) (Ukena and Tsutsui, 2005; Tsutsui and Ukena, 2006) and 26RFa/QRFP (Ukena et al., 2011, 2013, 2014b), in the avian brain. It has been demonstrated that these neuropeptides play important roles in the feeding behavior of birds and mammals (Tsutsui and Ubuka, 2016; Ukena et al., 2011, 2013, 2014b). Based on these research backgrounds, we were interested in feeding behavior and growth control processes.

Energy homeostasis, including food intake, energy expenditure, and body mass gain, is closely related to the development and growth process in animals. In the mammalian brain, it is well known that the arcuate nucleus (Arc) of the hypothalamus is one of the centers that control feeding and body mass (Schwartz and Porte, 2005; Morton et al., 2006, 2014). The Arc produces several neuropeptides, such as neuropeptide Y (NPY) and α-melanocyte-stimulating hormone (α-MSH), which are potent orexigenic and anorexigenic factors, respectively (Schwartz and Porte, 2005; Morton et al., 2006, 2014). It is well known that agouti-related protein (AgRP) is produced in NPY neurons, and that α-MSH is derived from the precursor of proopiomelanocortin (POMC). It has been reported that feeding-related neuropeptides have similar and differential effects in mammals and birds (Tachibana and Tsutsui, 2016). In addition, other unknown neuronal factors may take part in the regulation of feeding and energy metabolism. Therefore, we chose to study the chicken mediobasal hypothalamus, which includes the infundibular nucleus (IN), corresponding to the mammalian Arc, to identify previously unknown neuropeptides that affect those processes in birds.

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2. Identification of a cDNA encoding neurosecretory protein GL

To find novel neuropeptides, we utilized a cDNA subtractive screening method and searched for novel cDNAs that encoded precursors of neuropeptides. In general, the precursor protein (preproprotein) of a secretory peptide consists of an N-terminal signal peptide sequence, a bioactive secretory peptide sequence, and a dibasic amino acid sequence (Lys/Arg-Lys/Arg) in the internal sequence. In addition, approximately half of all bioactive peptides possess C-terminal amidation (-$CONH_2$), which is considered necessary for receptor binding and/or protection against degradative enzymes (Kim and Seong, 2001). For C-terminal amidation, the Gly residue serves as the amidation donor after the bioactive peptide sequence. Our initial purpose of this study was to discover a cDNA that encodes a novel precursor protein with these structural features. We expected that the cDNA would be a previously unknown neuropeptide precursor.

The cDNA subtractive screening yielded 596 highly expressed cDNA clones in the mediobasal hypothalamus of chicks. We determined the partial nucleotide sequences by DNA sequencing. Finally, we selected one cDNA highly expressed in the mediobasal hypothalamus for further study. The nucleotide sequence of the open reading frame was read and comprised of 549 bp. The deduced protein consisted of 182 amino acid residues and did not belong to any of the known protein families in animals (Fig. 1) (Ukena et al., 2014a). Analysis of the N-terminal sequence of the deduced protein with the SignalP program (www.cbs.dtu.dk/services/SignalP/) revealed the presence of a typical hydrophobic 32-amino acid signal peptide (Fig. 1). The predicted small protein of 80 amino acid residues was flanked at the C-terminus by a dibasic Lys-Arg (KR) motif, which constitutes a potential proteolytic processing site (Fig. 1). The precursor protein also possessed a Gly residue at its C-terminal end that contributed to amidation (Fig. 1). Because the predicted C-terminal amino acid sequence of the small protein was Gly-Leu-NH_2, the small protein was named neurosecretory protein GL (NPGL) (Ukena et al., 2014a). NPGL contains two Cys residues, suggesting an intramolecular disulfide bond bridge (Figs. 1 and 2).

Subsequently, we performed a TBLASTN search on the genome database using the amino acid sequence of the NPGL precursor. We identified an orthologous gene in humans and rats (Ukena et al., 2014a). Furthermore, the *Npgl* gene is conserved in other animals such as the mouse, western painted turtle, western clawed frog, and takifugu, and the alignment of the precursor protein sequences deduced from the orthologous gene in these animals is shown in Fig. 1. The precursor proteins seem to be coded in

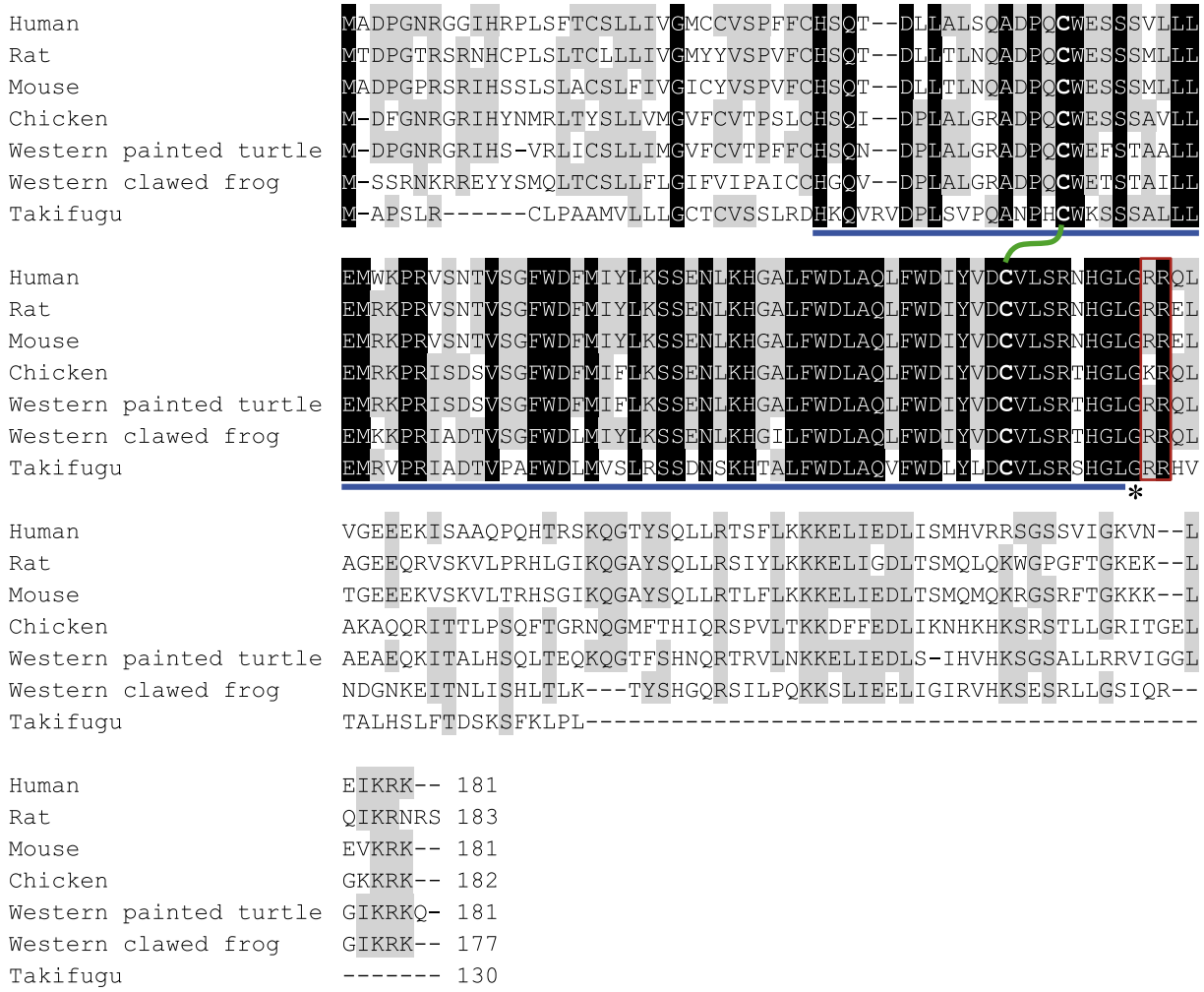

Fig. 1. Amino acid sequence alignment of neurosecretory protein GL (NPGL) precursor proteins deduced from cDNA or genomic DNA sequences of human (*Homo sapiens*), rat (*Rattus norvegicus*), mouse (*Mus musculus*), chicken (*Gallus gallus*), western painted turtle (*Chrysemys picta bellii*), western clawed frog (*Xenopus tropicalis*), and takifugu (*Takifugu rubripes*). Black and gray boxes highlight fully conserved and highly conserved amino acids, respectively. Gaps, indicated by hyphens, were inserted to optimize the sequence alignment. The predicted mature small proteins (NPGL) are underlined by blue lines. The disulfide bridge between the two conserved Cys (C) residues is indicated by a green line. The Gly (G) C-terminal amidation signal and the dibasic processing site [Arg-Arg (RR) or Lys-Arg (KR)] are indicated by an asterisk and red box, respectively.

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