MOLECULAR EVOLUTION OF GPCRS

26Rfa/GPR103

Kazuyoshi Ukena[1], Tomohiro Osugi[2,†], Jérôme Leprince[3], Hubert Vaudry[3] and Kazuyoshi Tsutsui[2]

[1]Section of Behavioral Sciences, Graduate School of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739-8521, Japan [2]Laboratory of Integrative Brain Sciences, Department of Biology, Center for Medical Life Science of Waseda University, Waseda University, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan [3]INSERM U982, Institute for Research and Innovation in Biomedicine (IRIB), Normandy University, 76821 Mont-Saint-Aignan, France
[†]T Osugi is now at Suntory Foundation for Life Sciences, Bioorganic Research Institute, Osaka 618-8503, Japan

Correspondence should be addressed to K Tsutsui
Email
k-tsutsui@waseda.jp

Abstract

Neuropeptides possessing the Arg-Phe-NH_2 (RFamide) motif at their C-termini (designated as RFamide peptides) have been characterized in a variety of animals. Among these, neuropeptide 26RFa (also termed QRFP) is the latest member of the RFamide peptide family to be discovered in the hypothalamus of vertebrates. The neuropeptide 26RFa/QRFP is a 26-amino acid residue peptide that was originally identified in the frog brain. It has been shown to exert orexigenic activity in mammals and to be a ligand for the previously identified orphan G protein-coupled receptor, GPR103 (QRFPR). The cDNAs encoding 26RFa/QRFP and QRFPR have now been characterized in representative species of mammals, birds, and fish. Functional studies have shown that, in mammals, the 26RFa/QRFP–QRFPR system may regulate various functions, including food intake, energy homeostasis, bone formation, pituitary hormone secretion, steroidogenesis, nociceptive transmission, and blood pressure. Several biological actions have also been reported in birds and fish. This review summarizes the current state of identification, localization, and understanding of the functions of 26RFaQRFP and its cognate receptor, QRFPR, in vertebrates.

Key Words
- 26RFa/QRFP
- food intake
- G protein-coupled receptor
- hypothalamus
- neuropeptide

Journal of Molecular Endocrinology
(2014) **52**, T119–T131

Journal of Molecular Endocrinology

Introduction

Neuropeptides that possess the Arg-Phe-NH_2 motif at their C-termini (i.e., RFamide peptides) have been characterized both in invertebrates and vertebrates. The first RFamide peptide to be identified was the cardioexcitatory peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide), which was isolated from the ganglia of the Venus clam *Macrocallista nimbosa* (Price & Greenberg 1977). Since then, a number of RFamide peptides have been identified in invertebrates, where these peptides seem to act as neurotransmitters and neuromodulators (for review, see Walker *et al.* (2009)).

A number of immunohistochemical studies that used antisera against FMRFamide suggested that the nervous system of vertebrates also contained neuropeptides immunologically related to FMRFamide (Raffa 1988, Vallarino *et al.* 1991, 1994, 1995, Rastogi *et al.* 2001). In fact, several neuropeptides harboring the RFamide sequence at their C-terminal end have been characterized in the brain of various vertebrates. In the past, the existence of five groups within the RFamide peptide family has been recognized in vertebrates, namely

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd.
This paper is one of eight papers that form part of a thematic review section on the Molecular Evolution of GPCRs. The Guest Editor for this section was Hubert Vaudry, European Institute for Peptides Research, University of Rouen, France. He was not involved in the handling of this paper, on which he is listed as an author.

the neuropeptide FF (NPFF) group, the prolactin-releasing peptide (PrRP) group, the gonadotropin-inhibitory hormone (GnIH) group, the kisspeptin group, and the 26RFa/QRFP group (for reviews, see Ukena & Tsutsui (2005), Bruzzone *et al*. (2006), Osugi *et al*. (2006), Tsutsui & Ukena (2006), Tsutsui (2009), Tsutsui *et al*. (2010*a*,*b*), Chartrel *et al*. (2011), Leprince *et al*. (2013); Fig. 1). These RFamide peptides have been shown to exert important neuroendocrine, behavioral, sensory, and autonomic functions (for reviews, see Chartrel *et al*. 2002, 2006*a*, Ukena & Tsutsui 2005, Tsutsui & Ukena 2006). Among these vertebrate RFamide peptides, NPFF is well documented as a morphine modulatory peptide (Panula *et al*. 1999). In addition, GnIH and kisspeptin appear to play key roles in the regulation of the reproductive axis (Tsutsui *et al*. 2010*b*). This review summarizes the current state of knowledge on the molecular evolution and functions of 26RFa/QRFP, the latest member of the RFamide peptide family to be discovered in vertebrates, and of its cognate receptor, QRFPR. This review also indicates future directions in this research field.

Unity and diversity of the structure of 26RFa/QRFP in vertebrates

The 26-amino acid residue RFamide peptide, 26RFa/QRFP, was identified for the first time in the brain of an amphibian species (Chartrel *et al*. 2003). An antibody against the RFamide motif was used to screen, by RIA, peptide fractions purified from a brain extract of the European green frog (*Rana esculenta*). After HPLC purification, the sequence of the isolated substance was analyzed by mass spectrometry MS/MS fragmentation; it turned out to be a 26-amino acid peptide possessing the RFamide motif at its C-terminus, namely VGTALGSLAEELNGYNRKKGGFSFRFamide. This neuropeptide had not been reported in any animals previously and was designated as 26RFa (Fig. 2A; Chartrel *et al*. 2003).

The amino acid sequence of frog 26RFa was employed to identify the cDNA encoding the counterpart of 26RFa in rat and humans (Chartrel *et al*. 2003). Concurrently, two other research groups independently identified 26RFa/QRFP precursors using a bioinformatic approach in the rat, mouse, bovine, and human genomes and paired 26RFa/QRFP with a previously identified orphan G protein-coupled receptor (GPCR), GPR103, also known as AQ27 or SP9155 (Fukusumi *et al*. 2003, Jiang *et al*. 2003; Fig. 2B). *GPR103* has thus been renamed *QRFPR* by the HUGO Gene Nomenclature Committee (http://www.genenames.org/). The mature 43-amino acid residue RFamide peptide was identified from the culture medium of CHO cells that expressed the human peptide precursor (Fukusumi *et al*. 2003). As the N-terminal amino acid was pyroglutamic acid, this RFamide peptide was also named pyroglutamylated RFamide peptide (QRFP; Fukusumi *et al*. 2003). Subsequently, the cDNAs encoding the 26RFa/QRFP precursors have been characterized in goldfish (Liu *et al*. 2009), quail (Ukena *et al*. 2010), chicken (Ukena *et al*. 2010), and zebra finch (Tobari *et al*. 2011) (Fig. 2B). Although the *26RFa/qrfp* cDNA has not been characterized in the European green frog, the corresponding sequence in the African clawed frog (*Xenopus tropicalis*) is present in the database (Fig. 2B). Furthermore, homologous sequences have been listed in the genome database of reptilian (lizard) and fish (stickleback, medaka, fugu, and zebrafish) species (Liu *et al*. 2009). These data have revealed the existence of the 26RFa/QRFP-encoding gene in representative species of the whole vertebrate phyla, including fish, amphibians, reptilians, birds, and mammals (Chartrel *et al*. 2011, Ukena *et al*. 2011).

As there are several monobasic processing sites in the 26RFa/QRFP precursor protein, alternative cleavage may yield various N-terminally elongated forms of 26RFa/QRFP (Chartrel *et al*. 2006*b*, 2011). HPLC analysis combined with RIAs indicated the existence of both 26- and 43-amino acid residue RFamide peptide-like immunoreactivities in the hypothalamus and spinal cord of humans (Bruzzone *et al*. 2006). Indeed, an N-terminally extended peptide of 43 residues, called 43RFa or QRFP, has been characterized in rat brain extracts, as well as in PC12 cells and the culture medium of CHO cells that express the human precursor, as described above (Fukusumi *et al*. 2003, Bruzzone *et al*. 2006, Takayasu *et al*. 2006; Fig. 2A). The human and *Xenopus* 26RFa/QRFP precursors may also generate a nine-amino acid peptide, termed 9RFa, located upstream of 26RFa/QRFP (Fig. 2B). However, 9RFa has not been detected in tissue extracts to date. Structure–activity relationship studies have revealed that the synthetic C-terminal heptapeptide ($26RFa_{20–26}$; GGFSFRFamide) is responsible for the biological activity of 26RFa/QRFP (Le Marec *et al*. 2011, Neveu *et al*. 2012). A reverse pharmacological study has demonstrated that 26RFa/QRFP is a natural ligand for the previously identified orphan receptor, GPR103 (QRFPR), as described below (Fukusumi *et al*. 2003, Jiang *et al*. 2003, Takayasu *et al*. 2006).

As reported above, the mature forms of 26RFa/QRFP have been identified in the brains of amphibians and mammals (Chartrel *et al*. 2003, Bruzzone *et al*. 2006, Takayasu *et al*. 2006), but, until recently, the existence of 26RFa/QRFP has not been investigated in birds.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

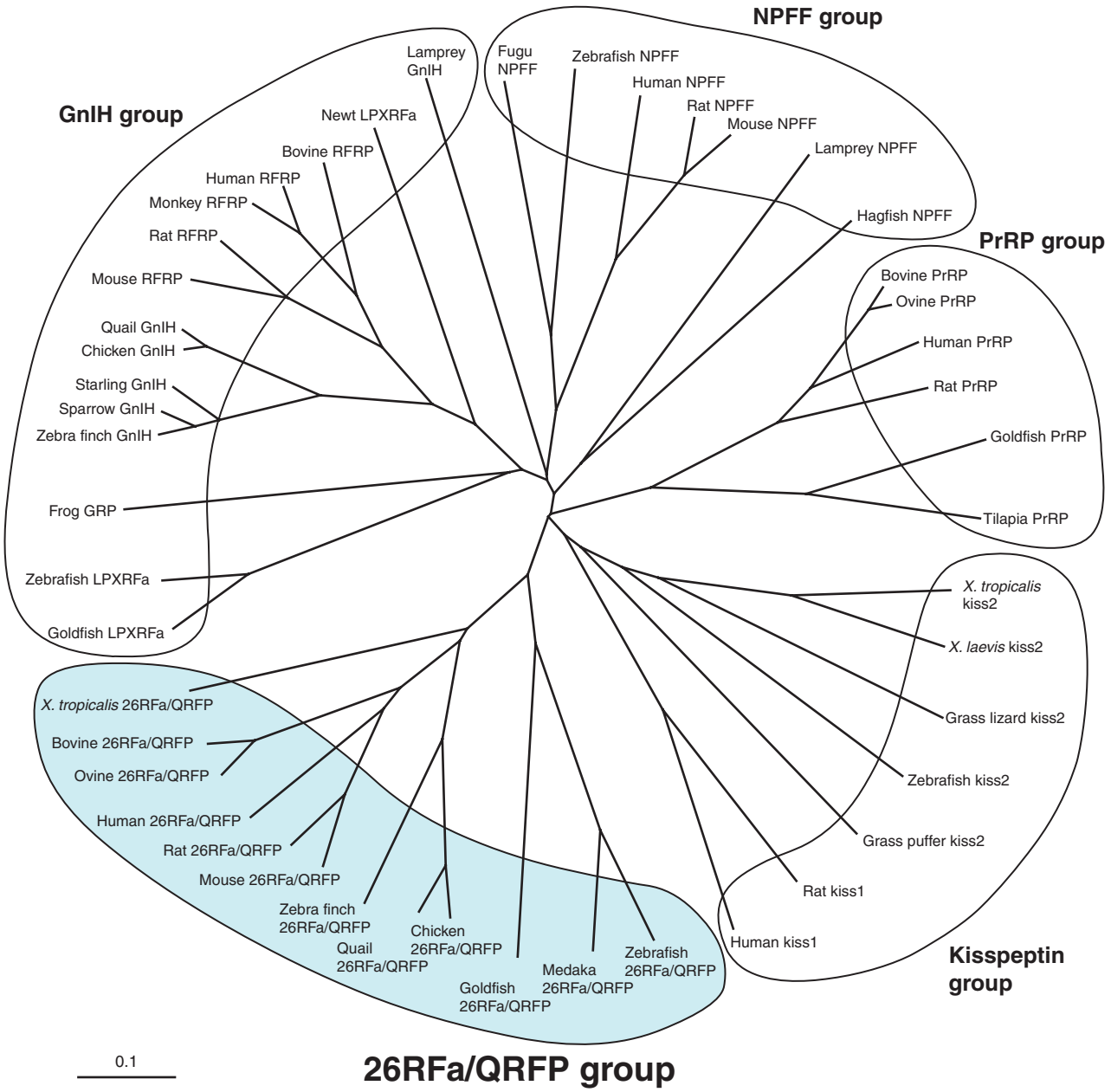

Figure 1

Phylogenetic tree of the RFamide peptide family in vertebrates. Studies over the past decade have demonstrated that the brain of vertebrates produces a variety of RFamide peptides. To date, five groups have been identified within this family: the neuropeptide FF (NPFF) group, the prolactin-releasing peptide (PrRP) group, the gonadotropin-inhibitory hormone (GnIH) group, the kisspeptin group, and the 26RFa/QRFP group.

Among the RFamide peptide family, only the GnIH group had been found in avian at the time we started this study (for reviews, see Ukena & Tsutsui 2005, Tsutsui & Ukena 2006, Tsutsui 2009, Tsutsui *et al.* 2010*a*,*b*). We therefore looked for *26RFa/QRFP* in the avian brain and found the presence of a gene encoding the 26RFa/QRFP precursor in chicken after searching the genomic database. Subsequently, the cDNA of the 26RFa/QRFP precursor was sequenced in the quail hypothalamus (Ukena *et al.* 2010). The quail precursor protein demonstrates 88% overall similarity with the chicken sequence, 47% with the corresponding human sequence, and 40% with the rat sequence (Ukena *et al.* 2011). A Lys-Arg dibasic cleavage site is present in the C-terminal region of the quail and

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

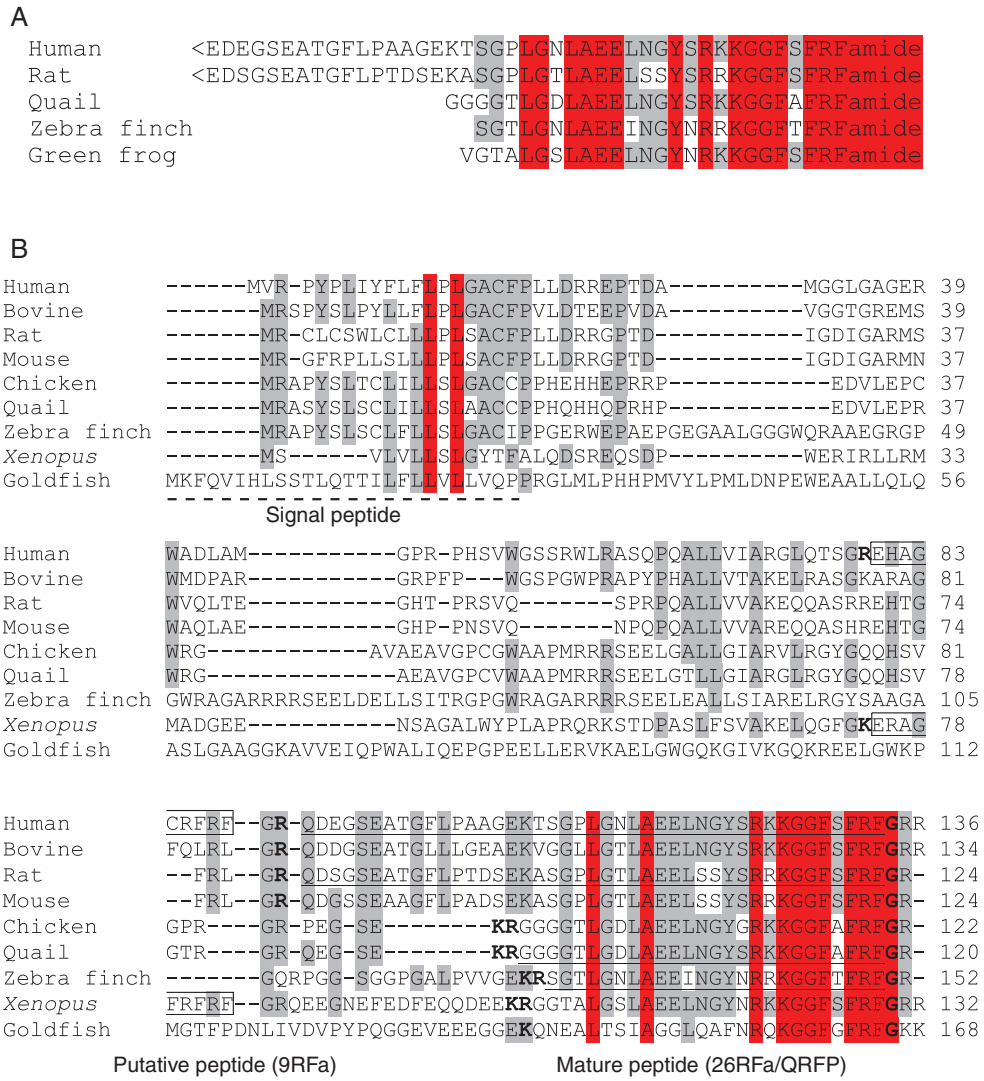

Figure 2

Alignments of the amino acid sequences of identified 26RFa/QRFP peptides (A) and their precursor proteins (B) deduced from mammalian (human, bovine, rat, and mouse), avian (chicken, quail, and zebra finch), amphibian (*Xenopus*), and fish (goldfish) cDNAs. The predicted signal peptide sequences are underlined with a dashed line. <E represents pyroglutamic acid. The positions of identified mature peptides in the precursor proteins are underlined with solid lines. The human and *Xenopus* 26RFa/QRFP precursors may also generate a nine-amino acid peptide, termed 9RFa (boxed). Fully conserved amino acids are highlighted with red boxes and highly conserved amino acids with gray boxes respectively. The Lys (K)-Arg (R) dibasic processing sites in birds and *Xenopus*, the single Arg (R) putative processing sites in mammals and fish, and the Gly (G) C-terminal amidation signals are shown in bold. Gaps marked by hyphens were inserted to optimize homology. The GenBank accession numbers of these sequences are as follows: human 26RFa/QRFP, NP_937823; bovine 26RFa/QRFP, NP_937865; rat 26RFa/QRFP, NP_937843; mouse 26RFa/QRFP, NP_906269; chicken 26RFa/QRFP, XP_001235089; quail 26RFa/QRFP, BAI81890; zebra finch 26RFa/QRFP, BAK32798; *Xenopus tropicalis* 26RFa/QRFP, XP_002936227; and goldfish 26RFa/Qrfp, ACI46681.

chicken precursor sequences, but not in that of mammalian sequences (Fig. 2B). This indicates that the mature peptide consists of 27 amino acid residues in quail and chicken, unlike the 26 residues in the amphibian 26RFa/QRFP sequence (Chartrel *et al*. 2003). In fact, MS analysis combined with immunoaffinity purification has revealed that the 27-amino acid sequence corresponds to the mature form of the peptide in the quail hypothalamus, indicating that the peptide is actually produced from the precursor in the hypothalamus (Ukena *et al*. 2010; Fig. 2A). More recently, a *26RFa/QRFP* ortholog, consisting of 25 amino acids, and the related cDNA have been characterized in the brain of zebra finch (Tobari *et al*. 2011; Fig. 2).

Synteny analysis of the *26RFa/QRFP* gene revealed that the chromosomal region encompassing the *26RFa/QRFP* gene is highly conserved from amphibians to

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

human. Indeed, all these regions contain paralogs of several other genes and thus clearly constitute a paralogon (Fig. 3). However, this paralogon has not been preserved in fish (Fig. 3), possibly because of the specific genome duplication and rearrangements that have occurred during the evolution in the fish lineage. To date, the existence of *26RFa/qrfp* gene in coelacanth and lamprey is still unclear (Fig. 3).

Comparative aspects of biological actions of 26RFa/QRFP in vertebrates

Mammals

The mRNAs encoding 26RFa/QRFP and its cognate receptor QRFPR are highly expressed in the dorsolateral and mediobasal hypothalamic areas of rodents (Chartrel *et al*. 2003, Takayasu *et al*. 2006, Bruzzone *et al*. 2007). These two areas are known to be involved in the regulation of energy homeostasis. In a similar way, in human, 26RFa/QRFP-producing cells are localized in the paraventricular and ventromedial nuclei of the hypothalamus (Bruzzone *et al*. 2006), which are also known to regulate food intake. Indeed, i.c.v. injection of 26RFa/QRFP has been demonstrated to stimulate food intake in rodents (Chartrel *et al*. 2003, Do Régo *et al*. 2006, Moriya *et al*. 2006, Takayasu *et al*. 2006, Primeaux *et al*. 2008, 2013, Lectez *et al*. 2009, Primeaux 2011).

In addition to its orexigenic effects, 26RFa/QRFP has been reported to exert a wide range of biological actions (Fig. 4). In an earlier report, i.v. administration of 26RFa/QRFP was found to increase plasma aldosterone levels in a dose-dependent manner in rats (Fukusumi *et al*. 2003). Recently, it has been reported that 26RFa/QRFP and QRFPR are present in the human and rat adrenal gland and that 26RFa/QRFP stimulates corticosteroid secretion

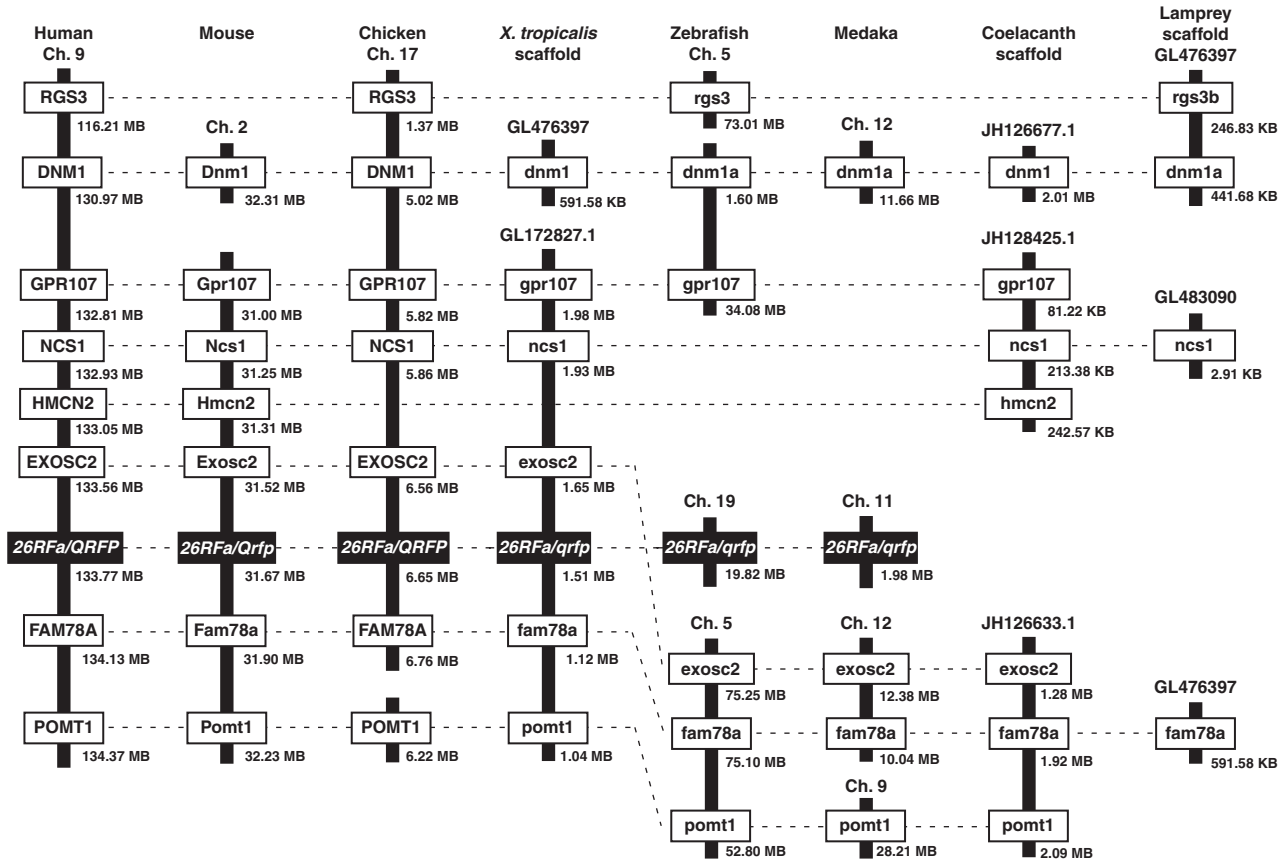

Figure 3

Synteny analysis around *26RFa/QRFP* gene loci. Orthologous or paralogous genes are linked by horizontal lines. The *26RFa/QRFP* genes are shown white in black boxes. The nucleotide position of each gene on the chromosome is shown under each gene. The GenBank accession numbers of *26RFa/QRFP* genes are as follows: human *26RFa/QRFP*, AB109625.1; mouse *26RFa/Qrfp*, AB109628.1; chicken *26RFa/QRFP*, XM_001235088.2; *Xenopus tropicalis 26RFa/qrfp*, XM_004916673.1; zebrafish *26RFa/qrfp*, XP_00133883; medaka *26RFa/qrfp*, XP_004073955.1.

Published by Bioscientifica Ltd

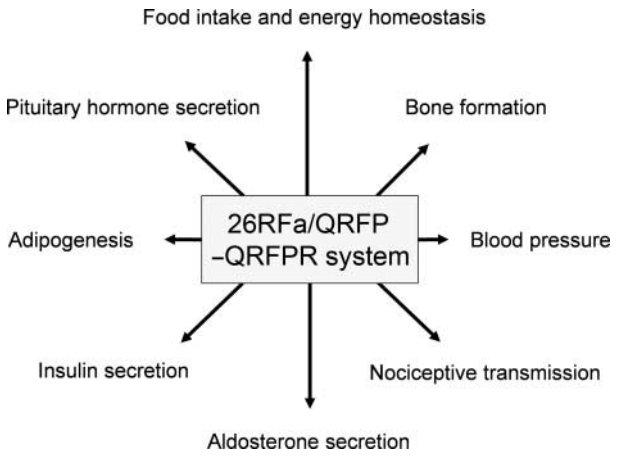

Figure 4
Demonstrated biological actions of 26RFa/QRFP–QRFPR system in vertebrates. Both 26RFa/QRFP and QRFPR have been found to exert a wide array of biological activities.

by human adrenocortical cells (Ramanjaneya *et al*. 2013). In the rat pancreas, glucose-evoked insulin secretion is reduced by perfusion of 26RFa/QRFP (Egido *et al*. 2007). In the adipocyte cell line 3T3-L1, 26RFa/QRFP inhibits isoproterenol-induced lipolysis (Malumba *et al*. 2010). As 3T3-L1 cells express the QRFPR-encoding gene, it appears that 26RFa/QRFP may act in an autocrine/paracrine manner to regulate adipogenesis (Malumba *et al*. 2010). According to Alonzeau *et al*. (2013), 26RFa/QRFP is also expressed in human prostate cancer and stimulates the neuroendocrine differentiation and migration of cancer cells. Administration of 26RFa/QRFP in the brain increases plasma luteinizing hormone (LH) levels in both sexes in rat (Navarro *et al*. 2006, Patel *et al*. 2008) and stimulates prolactin and growth hormone secretion in male rhesus monkeys (Qaiser *et al*. 2012, Wahab *et al*. 2012). Intrathecally administered 26RFa/QRFP induces analgesic effects in rat under formalin and carrageenan tests (Yamamoto *et al*. 2008). Central injection of 26RFa/QRFP in mice causes a rise in blood pressure and heart rate (Takayasu *et al*. 2006). Mice deficient in the receptor for 26RFa/QRFP (*Qrfpr*) suffer from osteopenia (Baribault *et al*. 2006). This observation indicates that 26RFa/QRFP plays a major role in bone formation, via QRFPR that is expressed in bone (Baribault *et al*. 2006).

Non-mammalian vertebrates

In goldfish, quantitative RT-PCR analysis demonstrated high expression of *26RFa/qrfp* mRNA in the hypothalamus, optic tectum–thalamus, and testis. The expression of *26RFa/qrfp* mRNA in the hypothalamus is augmented at 4 days after food deprivation (Liu *et al*. 2009). In addition, serum LH levels are significantly increased at 1 h, but not at 3 and 6 h after i.p. injection of 26RFa/QRFP (Liu *et al*. 2009). As 26RFa/Qrfp has no effect on LH release from pituitary cells in primary culture, it is thought that, in fish, the peptide may stimulate the gonadotropic axis by acting exclusively at the hypothalamic level. These results suggest that 26RFa/Qrfp regulates energy homeostasis and the hypothalamic–pituitary–gonadal axis in fish, as also observed in mammals.

In birds, the expression of *26RFa/QRFP* mRNA in the quail brain has been investigated in different brain regions, i.e., the cerebrum, diencephalon, mesencephalon, and cerebellum, by quantitative PCR analysis. A high level of expression of *26RFa/QRFP* mRNA is present in the diencephalon, including the hypothalamus, while *26RFa/QRFP* mRNA is almost undetectable in other brain regions (Ukena *et al*. 2010). In colchicine-treated birds (quail and chicken), 26RFa/QRFP-immunoreactive cell bodies were found only in the anterior hypothalamic nucleus in the diencephalon (Ukena *et al*. 2010). Furthermore, *in situ* hybridization has shown specific expression of *26RFa/QRFP* mRNA in the anterior hypothalamic nucleus in the chick brain, and the distribution of *26RFa/QRFP* mRNA-containing perikarya clearly matches with that of 26RFa/QRFP-immunoreactive neurons (Ukena *et al*. 2010). In the zebra finch, *in situ* hybridization analysis has revealed that expression of *26RFa/QRFP* mRNA is localized to the anterior–medial hypothalamic area, the ventromedial nucleus of the hypothalamus, and the lateral hypothalamic area (Tobari *et al*. 2011). These neuroanatomical data suggest that, in birds, 26RFa/QRFP produced in the hypothalamus participates in the control of feeding behavior, as shown previously in rodents (Chartrel *et al*. 2006*b*, Do Régo *et al*. 2006, Moriya *et al*. 2006, Primeaux *et al*. 2008).

To assess the above speculation, the effect of central injection of 26RFa/QRFP has been surveyed in both broiler and layer chick lines. I.c.v. injection of 26RFa/QRFP stimulates feeding behavior in broiler chicks, but not in layer chicks (Ukena *et al*. 2010). It is likely that the different effects in these two chick lines can be explained by the following reports. It has been demonstrated that the effect of 26RFa/QRFP on feeding behavior in rodents differs according to the energy status and/or the species (Primeaux *et al*. 2013). Although 26RFa/QRFP hardly affects food intake in normally fed rats (Fukusumi *et al*. 2003, Kampe *et al*. 2006, Patel *et al*. 2008), at least under a low-fat diet (Primeaux *et al*. 2008), 26RFa/QRFP induces a

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

marked orexigenic effect in mice and food-restricted rats (Chartrel *et al*. 2003, 2005, Do Régo *et al*. 2006, Moriya *et al*. 2006, Takayasu *et al*. 2006, Lectez *et al*. 2009). In addition, it has been demonstrated that 26RFa/QRFP selectively increases the intake of a high-fat diet in rats (Primeaux *et al*. 2008, 2013, Primeaux 2011). On the other hand, to determine the biologically active core of 26RFa/QRFP, the effect of a synthetic C-terminal octapeptide (26RFa-8; KGGFAFRFamide) of 26RFa/QRFP has been tested on feeding behavior of chicken. This C-terminal sequence is highly conserved from fish to mammals (Fig. 2). The synthetic C-terminal octapeptide, 26RFa-8, stimulates food intake in broiler chicks, but not in layer chicks, in much the same manner as the full-length peptide (Ukena *et al*. 2010). Consistent with this observation, a synthetic C-terminal heptapeptide of 26RFa/QRFP ($26RFa_{20–26}$; GGFSFRFamide) exerts an orexigenic effect in mice (Do Régo *et al*. 2006). In addition, $26RFa_{20–26}$ evokes a significant increase in serum LH levels in female rats (Navarro *et al*. 2006). Taken together, it appears that the C-terminal region of 26RFa/QRFP is responsible for the biological activity of the peptide. In addition to the chick data, it has been reported that central injection of 26RFa/QRFP in free-feeding male zebra finches stimulates food intake for 24 h, without a change in body mass (Tobari *et al*. 2011). These results also indicate that 26RFa/QRFP exerts an orexigenic activity in various avian species.

Comparative aspects of QRFPR in vertebrates

Mammals

In humans, 26RFa/QRFP has been found to be an endogenous ligand for the orphan receptor, GPR103 (QRFPR), which is a class A GPCR (Fukusumi *et al*. 2003, Jiang *et al*. 2003). QRFPR shares relatively high sequence similarity with other RFamide receptors, notably those for NPFF, PrRP, kisspeptin, and GnIH, and to a lesser extent with the other peptidergic receptors for neuropeptide Y (NPY), galanin, orexin, and cholecystokinin (Lee *et al*. 2001, Jiang *et al*. 2003). Surprisingly, 26RFa/QRFP displays a moderate affinity for NPFF2 (NPFFR2, the receptor for NPFF) and a low affinity for NPFF1 (NPFFR1, the receptor for GnIH) (Gouardères *et al*. 2007). In addition, QRFPR possesses several characteristic features of class A GPCRs, such as i) a disulfide bridge between the two Cys (C) residues located in the first and second extracellular loops (EL1 and EL2), ii) the existence of an Asp (D) residue within the second transmembrane domain (TM2) that seems to play a pivotal role in G protein coupling, iii) a conserved Glu (E)-Arg (R) doublet sequence at the N-terminal end of the second intracellular loop (IL2), and iv) three conserved residues, i.e., Phe (F), Pro (P) and Asn (N), within TM6 and TM7, which are crucial for receptor activation (Fig. 5). QRFPs with 26 and 43 amino acid residues bind to QRFPR with high affinity (EC_{50}=3.2 and 0.52 nM respectively) (Fukusumi *et al*. 2003, Jiang *et al*. 2003). It has also been demonstrated that 26- and 43-amino acid residue QRFPs inhibit cAMP formation with similar efficacy in *QRFPR*-transfected CHO cells (Fukusumi *et al*. 2003). Furthermore, 26RFa/QRFP markedly increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in a pertussis toxin-independent manner. These results suggest that QRFPR is coupled to a $G_{i/0}$ and/or to a G_q protein (Fukusumi *et al*. 2003). The affinity and potency of the C-terminal heptapeptide $26RFa_{20–26}$ (GGFSFRFamide) have been investigated and were found to be lower than those of 26RFa/QRFP. These data indicate that this heptapeptide is a relatively weak ligand for QRFPR (Fukusumi *et al*. 2003, Le Marec *et al*. 2011). Furthermore, it has been reported that 26RFa/QRFP enhances corticosteroid secretion in human adrenocortical cells by regulating key steroidogenic enzymes involving MAPK/PKC and Ca^{2+} signaling pathways via QRFPR (Ramanjaneya *et al*. 2013).

In contrast to humans, who only have a single QRFPR-encoding gene, two isoforms of the receptor for 26RFa/QRFP have been characterized in rodents. These 26RFa/QRFP receptor isoforms have been designated as QRFPR1 and QRFPR2 in rat and mouse (Kampe *et al*. 2006, Takayasu *et al*. 2006); 26RFa/QRFP stimulates inositol trisphosphate in rat QRFPR1 and QRFPR2 with similar efficacy (Kampe *et al*. 2006) and binds to mouse QRFPR1 and QRFPR2 with similar affinity (Takayasu *et al*. 2006).

The distribution of *QRFPR* mRNA and its peptide binding sites have been studied by *in situ* hybridization and autoradiography respectively. In rat, *Qrfpr* mRNA-containing cells are notably expressed in the midbrain, the pons, and the medulla oblongata, while 26RFa/QRFP-binding sites are widely distributed throughout the brain and spinal cord (Bruzzone *et al*. 2007). These results suggest that 26RFa/QRFP can bind to a receptor(s) other than QRFPR. Indeed, it has been found by competition experiments that 26RFa/QRFP interacts with NPFF2, the cognate receptor for NPFF (Bruzzone *et al*. 2007). The widespread distribution of 26RFa/QRFP-binding sites suggests that 26RFa/QRFP exerts multiple functions in the brain and spinal cord that are mediated

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

Figure 5

Alignment of the amino acid sequences of the G protein-coupled receptor for 26RFa/QRFP, QRFPR, in mammals (human and rat), birds (chicken and zebra finch), frog (*Xenopus*), and fish (zebrafish). Fully conserved amino acids are highlighted with green boxes and highly conserved amino acids with gray boxes. Putative transmembrane domains (TMD) are underlined. The disulfide bridge between the two Cys (C) residues located in the first and second extracellular loops is indicated by a line. The Asp (D) residue in TMD2 involved in G protein coupling, the conserved Glu (E)-Arg (R) residues in the second intracellular loop, and the conserved Phe (F), Pro (P), and Asn (N) residues in TMD6 and TMD7 are represented by colored letters. A hyphen has been inserted to obtain optimal homology. The GenBank accession numbers of these sequences are as follows: human QRFPR, NP_937822; rat QRFPR, NP_937842; chicken QRFPR, NP_001120642; zebra finch QRFPR, NP_001243137; *Xenopus tropicalis* QRFPR, NP_001072295; and zebrafish Qrfpr, XP_001920042.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

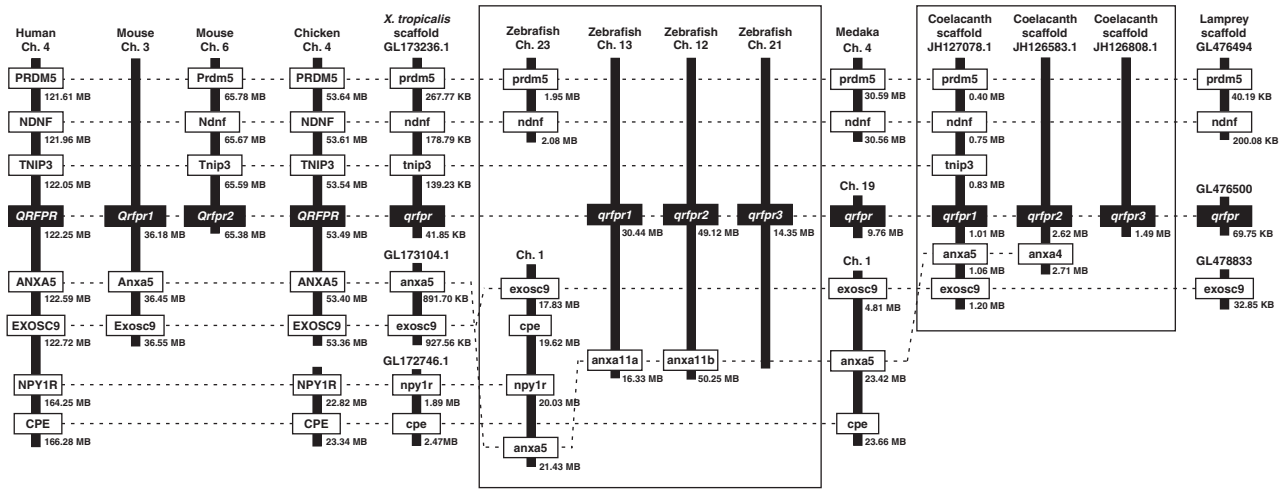

Figure 6

Synteny analysis around *QRFPR* gene loci. Orthologous or paralogous genes are linked by horizontal lines. The *QRFPR* genes are shown white in black boxes. The nucleotide position of each gene on the chromosome is shown under each gene. The GenBank accession numbers of *QRFPR* genes are as follows: human *QRFPR*, JF810892.1; mouse *Qrfpr1*, BC096610.1; chicken *QRFPR*, NM_001127170.1; *Xenopus tropicalis qrfpr*, NM_001078827.1; and medaka *qrfpr*, XP_004080459.1. Ensembl genome database accession numbers are as follows: mouse *Qrfpr2*, ENSMUSG00000029917; zebrafish *qrfpr1*, ENSDARG00000039349; zebrafish *qrfpr2*, ENSDARG00000068422; zebrafish *qrfpr3*, ENSDARG00000092652; coelacanth *qrfpr*, ENSLACG00000016226; and sea lamprey *qrfpr*, ENSPMAG00000005451. GENSCAN (http://genes.mit.edu/GENSCAN.html) was used to predict putative coelacanth Qrfpr3 precursor protein.

by at least two distinct receptors, QRFPR and NPFF2 (Bruzzone *et al*. 2007).

Non-mammalian vertebrates

In birds, the cDNAs encoding QRFPR have been characterized in the brain of chicken and zebra finch (Ukena *et al*. 2010, Tobari *et al*. 2011). The sequence of chicken QRFPR is highly similar to those of human and rat QRFPR (Fig. 5). The action of 26RFa/QRFP on chicken QRFPR has been studied by measuring $[Ca^{2+}]_i$ in HEK293T cells that had been transiently transfected with chicken *QRFPR*. In these cells, 26RFa/QRFP increases $[Ca^{2+}]_i$ in a dose-dependent manner, with an EC_{50} value of around 40 nM (Ukena *et al*. 2010). The mRNA of QRFPR is widely expressed in chicken and zebra finch brains and the highest concentration of mRNA is observed in the diencephalon (Ukena *et al*. 2010, Tobari *et al*. 2011). As the mRNA of QRFPR is expressed in the brain outside the diencephalon in chicken, as it is in rat (Bruzzone *et al*. 2007), 26RFa/QRFP may exert multiple functions in addition to regulating food intake (Ukena *et al*. 2010).

Synteny analysis has revealed the existence of species-specific paralogous genes of QRFPR in mouse, zebrafish and coelacanth (Fig. 6). These paralogous genes may have emerged along with the species-specific gene or genome duplications that occurred during the course of vertebrate evolution. Phylogenetic analysis data are consistent with synteny analysis (Fig. 7). Although there are homologous sequences to *QRFPR* in the genome database of *Xenopus*, zebrafish, coelacanth and lamprey (Figs 5 and 6), *Qrfpr* has been studied only in mammals and birds. Further characterization of QRFPR is thus needed to determine the functional significance of the 26RFa/QRFP–QRFPR system in other vertebrate phyla, such as reptilians, amphibians, and fish.

Conclusions and future directions

The neuropeptide 26RFa/QRFP belongs to the most recently identified group of the RFamide peptide family and was first identified in the brain of the European green frog. Subsequently, the cDNAs encoding the 26RFa/QRFP precursors have been characterized in various animals, including goldfish, quail, chicken, zebra finch, mouse, rat, bovine, and humans, and these analyses have shown the existence of the 26RFa/QRFP-encoding gene in representative species of the vertebrate phylum. In mammals, 26RFa/QRFP has been found to be a high-affinity endogenous ligand for the previously identified orphan GPCR, GPR103 (QRFPR). In rodents and monkeys, 26RFa/QRFP exerts diverse biological actions, including regulation of food intake and energy homeostasis, hormone secretion, nociception, and bone formation.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

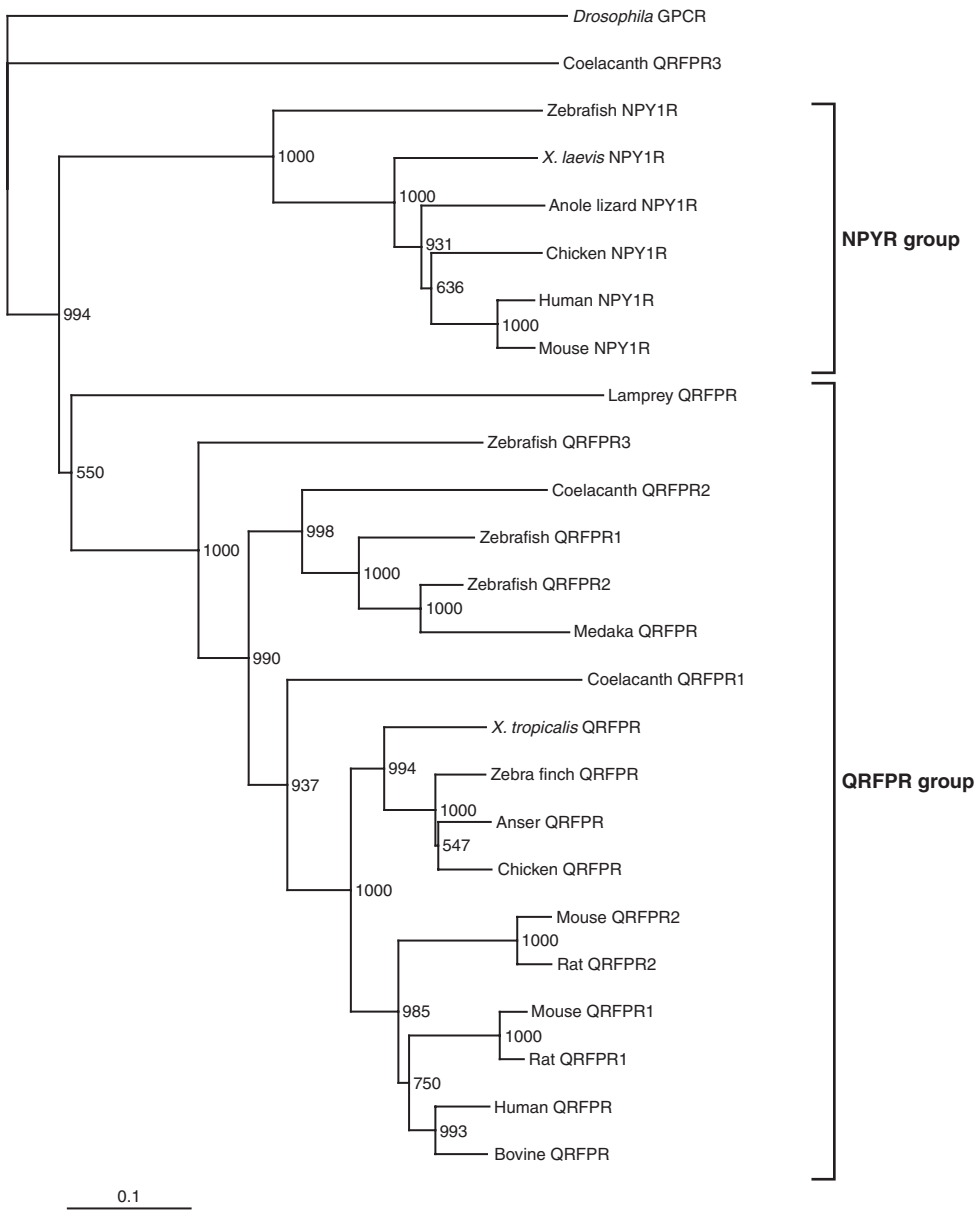

Figure 7

Phylogenetic analysis of QRFPR precursor proteins. *Drosophila melanogaster* peptide GPCR was used as an outgroup. NPY receptors are included in the phylogenetic tree as a reference group of vertebrate GPCR. Scale bar refers to a phylogenetic distance of 0.1 nucleotide substitutions per site. Numbers on the branches indicate bootstrap percentage following 1000 replications in constructing the tree. The GenBank accession numbers of the *NPY1R* genes are as follows: human *NPY1R*, NM_000909; mouse *Npy1r*, NM_010934; chicken *NPY1R*, NM_001031535; anole lizard *NPY1R*, XM_003221700; *Xenopus laevisnpy1r*, NM_001085879; zebrafish *npy1r*, NM_001102391; and *Drosophila melanogaster* peptide GPCR, AY217746.1.

Recently, the mature sequences of 26RFa/QRFP have been identified by structural analysis in quail and zebra finch. In birds, as in mammals, 26RFa/QRFP-producing neurons are only located in the hypothalamus, while QRFPR is widely distributed throughout the brain. In birds, 26RFa/QRFP also exerts an orexigenic action, as it does in rodents, and a similar effect of 26RFa/QRFP has been suggested in fish, because of upregulation of *26RFa/qrfp* mRNA by a negative energy state. Thus, the structure, distribution pattern, and biological actions of the 26RFa/QRFP–QRFPR system have been conserved across the vertebrate phylum, from fish to mammals. However, further studies are clearly required to

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

fully elucidate the molecular evolution and functional significance of the 26RFa/QRFP–QRFPR pair in vertebrates. In particular, *in vitro* and *in vivo* studies on development, morphogenesis, and behavior in non-mammalian model organisms, such as *Xenopus* and zebrafish, should bring to light previously unknown physiological actions of the 26RFa/QRFP–QRFPR system. Recent studies have shown that a number of neuropeptide/GPCR pairs initially discovered in vertebrates/deuterostomes actually possess homologs in protostomes (Sherwood *et al*. 2006, Roch *et al*. 2011, Frooninckx *et al*. 2012, Grimmelikhuijzen & Hauser 2012, Mirabeau & Joly 2013). It would thus be interesting to look for the existence of 26RFa/QRFP and/or QRFPR orthologs in representative species of protostomes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan (20770056 and 22687004 to K U and 18107002, 22132004, and 22227002 to K T), the Toray Science Foundation to K U, the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry to K U, and a France–Japan exchange program (INSERM-JSPS) to K T and H V.

References

Alonzeau J, Alexandre D, Jeandel L, Courel M, Hautot C, El Yamani FZ, Gobet F, Leprince J, Magoul R, Amarti A *et al*. 2013 The neuropeptide 26RFa is expressed in human prostate cancer and stimulates the neuroendocrine differentiation and the migration of androgeno-independent prostate cancer cells. *European Journal of Cancer* **49** 511–519. (doi:10.1016/j.ejca.2012.05.028)

Baribault H, Danao J, Gupte J, Yang L, Sun B, Richards W & Tian H 2006 The G-protein-coupled receptor GPR103 regulates bone formation. *Molecular and Cellular Biology* **26** 709–717. (doi:10.1128/MCB.26.2.709-717.2006)

Bruzzone F, Lectez B, Tollemer H, Leprince J, Dujardin C, Rachidi W, Chatenet D, Baroncini M, Beauvillain JC, Vallarino M *et al*. 2006 Anatomical distribution and biochemical characterization of the novel RFamide peptide 26RFa in the human hypothalamus and spinal cord. *Journal of Neurochemistry* **99** 616–627. (doi:10.1111/j.1471-4159.2006.04090.x)

Bruzzone F, Lectez B, Alexandre D, Jégou S, Mounien L, Tollemer H, Chatenet D, Leprince J, Vallarino M, Vaudry H *et al*. 2007 Distribution of 26RFa binding sites and GPR103 mRNA in the central nervous system of the rat. *Journal of Comparative Neurology* **503** 573–591. (doi:10.1002/cne.21400)

Chartrel N, Dujardin C, Anouar Y, Leprince J, Decker A, Clerens S, Do Régo JC, Vandesande F, Llorens-Cortes C, Costentin J *et al*. 2003 Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. *PNAS* **100** 15247–15252. (doi:10.1073/pnas.2434676100)

Chartrel N, Dujardin C, Leprince J, Desrues L, Tonon MC, Cellier E, Cosette P, Jouenne T, Simonnet G & Vaudry H 2002 Isolation, characterization, and distribution of a novel neuropeptide, Rana RFamide (R-RFa), in the brain of the European green frog *Rana esculenta*. *Journal of Comparative Neurology* **448** 111–127. (doi:10.1002/cne.10253)

Chartrel N, Bruzzone F, Dujardin C, Leprince J, Tollemer H, Anouar Y, Vallarino M, Costentin J & Vaudry H 2005 Identification of 26RFa from frog brain: a novel hypothalamic neuropeptide with orexigenic activity in mammals. *Annals of the New York Academy of Sciences* **1040** 80–83. (doi:10.1196/annals.1327.009)

Chartrel N, Tsutsui K, Costentin J & Vaudry H 2006*a* The RFamide-related peptides. In *Handbook of Biologically Actives Peptides*, pp 779–786. Eds AJ Kastin. Amsterdam: Elsevier.

Chartrel N, Bruzzone F, Leprince J, Tollemer H, Anouar Y, Do Régo JC, Ségalas-Milazzo I, Guilhaudis L, Cosette P, Jouenne T *et al*. 2006*b* Structure and functions of the novel hypothalamic RFamide neuropeptides R-RFa and 26RFa in vertebrates. *Peptides* **27** 1110–1120. (doi:10.1016/j.peptides.2005.06.035)

Chartrel N, Alonzeau J, Alexandre D, Jeandel L, Alvear-Perez R, Leprince J, Boutin J, Vaudry H, Anouar Y & Llorens-Cortes C 2011 The RFamide neuropeptide 26RFa and its role in the control of neuroendocrine functions. *Frontiers in Neuroendocrinology* **32** 387–397. (doi:10.1016/j.yfrne.2011.04.001)

Do Régo JC, Leprince J, Chartrel N, Vaudry H & Costentin J 2006 Behavioral effects of 26RFamide and related peptides. *Peptides* **27** 2715–2721. (doi:10.1016/j.peptides.2006.04.017)

Egido EM, Hernández R, Leprince J, Chartrel N, Vaudry H, Marco J & Silvestre RA 2007 26RFa, a novel orexigenic neuropeptide, inhibits insulin secretion in the rat pancreas. *Peptides* **28** 725–730. (doi:10.1016/j.peptides.2006.04.004)

Frooninckx L, Van Rompay L, Temmerman L, Van Sinay E, Beets I, Janssen T, Husson SJ & Schoofs L 2012 Neuropeptide GPCRs in *C. elegans*. *Frontiers in Endocrinology* **3** 167. (doi:10.3389/fendo.2012.00167)

Fukusumi S, Yoshida H, Fujii R, Murayama M, Komatsu H, Habata Y, Shintani Y, Hinuma S & Fujino M 2003 A new peptidic ligand and its receptor regulating adrenal function in rats. *Journal of Biological Chemistry* **278** 46387–46395. (doi:10.1074/jbc.M305270200)

Gouardères C, Mazarguil H, Mollereau C, Chartrel N, Leprince J, Vaudry H & Zajac JM 2007 Functional differences between NPFF1 and NPFF2 receptor coupling: high intrinsic activities of RFamide-related peptides on stimulation of [^{35}S]GTPγS binding. *Neuropharmacology* **52** 376–386. (doi:10.1016/j.neuropharm.2006.07.034)

Grimmelikhuijzen CJ & Hauser F 2012 Mini-review: the evolution of neuropeptide signaling. *Regulatory Peptides* **177** S6–S9. (doi:10.1016/j.regpep.2012.05.001)

Jiang Y, Luo L, Gustafson EL, Yadav D, Laverty M, Murgolo N, Vassileva G, Zeng M, Laz TM, Behan J *et al*. 2003 Identification and characterization of a novel RF-amide peptide ligand for orphan G-protein-coupled receptor SP9155. *Journal of Biological Chemistry* **278** 27652–27657. (doi:10.1074/jbc.M302945200)

Kampe J, Wiedmer P, Pfluger PT, Castaneda TR, Burget L, Mondala H, Kerr J, Liaw C, Oldfield BJ, Tschöp MH *et al*. 2006 Effect of central administration of QRFP(26) peptide on energy balance and characterization of a second QRFP receptor in rat. *Brain Research* **1119** 133–149. (doi:10.1016/j.brainres.2006.08.055)

Lectez B, Jeandel L, El-Yamani FZ, Arthaud S, Alexandre D, Mardargent A, Jégou S, Mounien L, Bizet P, Magoul R *et al*. 2009 The orexigenic activity of the hypothalamic neuropeptide 26RFa is mediated by the neuropeptide Y and proopiomelanocortin neurons of the arcuate nucleus. *Endocrinology* **150** 2342–2350. (doi:10.1210/en.2008-1432)

Lee DK, Nguyen T, Lynch KR, Cheng R, Vanti WB, Arkhitko O, Lewis T, Evans JF, George SR & O'Dowd BF 2001 Discovery and mapping of ten novel G protein-coupled receptor genes. *Gene* **275** 83–91. (doi:10.1016/S0378-1119(01)00651-5)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

Le Marec O, Neveu C, Lefranc B, Dubessy C, Boutin JA, Do-Régo JC, Costentin J, Tonon MC, Tena-Sempere M, Vaudry H *et al.* 2011 Structure–activity relationships of a series of analogues of the RFamide-related peptide 26RFa. *Journal of Medicinal Chemistry* **54** 4806–4814. (doi:10.1021/jm200418c)

Leprince J, Neveu C, Lefranc B, Guilhaudis L, Milazzo-Segalas I, Do Rego JC, Tena-Sempere M, Tsutsui K & Vaudry H 2013 26RFa. In *Handbook of Biologically Active Peptides* 2nd edn, pp 917–923. Ed A J Kastin. Amsterdam, The Netherlands: Elsevier.

Liu Y, Zhang Y, Li S, Huang W, Liu X, Lu D, Meng Z & Lin H 2009 Molecular cloning and functional characterization of the first non-mammalian 26RFa/QRFP orthologue in goldfish, *Carassius auratus*. *Molecular and Cellular Endocrinology* **303** 82–90. (doi:10.1016/j.mce.2009.01.009)

Malumba M, Jossart C, Granata R, Gallo D, Escher E, Ghigo E, Servant MJ, Marleau S & Ong H 2010 GPR103b functions in the peripheral regulation of adipogenesis. *Molecular Endocrinology* **24** 1615–1625. (doi:10.1210/me.2010-0010)

Mirabeau O & Joly JS 2013 Molecular evolution of peptidergic signaling systems in bilaterians. *PNAS* **110** E2028–E2037. (doi:10.1073/pnas.1219956110)

Moriya R, Sano H, Umeda T, Ito M, Takahashi Y, Matsuda M, Ishihara A, Kanatani A & Iwaasa H 2006 RFamide peptide QRFP43 causes obesity with hyperphagia and reduced thermogenesis in mice. *Endocrinology* **147** 2916–2922. (doi:10.1210/en.2005-1580)

Navarro VM, Fernández-Fernández R, Nogueiras R, Vigo E, Tovar S, Chartrel N, Le Marec O, Leprince J, Aguilar E, Pinilla L *et al.* 2006 Novel role of 26RFa, a hypothalamic RFamide orexigenic peptide, as putative regulator of the gonadotropic axis. *Journal of Physiology* **573** 237–249. (doi:10.1113/jphysiol.2006.106856)

Neveu C, Lefranc B, Tasseau O, Do Rego JC, Bourmaud A, Chan P, Bauchat P, Le Marec O, Chuquet J, Guilhaudis L *et al.* 2012 Rational design of a low molecular weight, stable, potent and long-lasting GPR103 aza-β3-pseudopeptide agonist. *Journal of Medicinal Chemistry* **55** 7516–7524. (doi:10.1021/jm300507d)

Osugi T, Ukena K, Sower SA, Kawauchi H & Tsutsui K 2006 Evolutionary origin and divergence of PQRFamide peptides and LPXRFamide peptides in the RFamide peptide family. Insights from novel lamprey RFamide peptides. *FEBS Journal* **273** 1731–1743. (doi:10.1111/j.1742-4658.2006.05187.x)

Panula P, Kalso E, Nieminen M, Kontinen VK, Brandt A & Pertovaara A 1999 Neuropeptide FF and modulation of pain. *Brain Research* **848** 191–196. (doi:10.1016/S0006-8993(99)02044-2)

Patel SR, Murphy KG, Thompson EL, Patterson M, Curtis AE, Ghatei MA & Bloom SR 2008 Pyroglutamylated RFamide peptide 43 stimulates the hypothalamic–pituitary–gonadal axis via gonadotropin-releasing hormone in rats. *Endocrinology* **149** 4747–4754. (doi:10.1210/en.2007-1562)

Price DA & Greenberg MJ 1977 Structure of a molluscan cardioexcitatory neuropeptide. *Science* **197** 670–671. (doi:10.1126/science.877582)

Primeaux SD 2011 GRFP in female rats: effects on high fat food intake and hypothalamic gene expression across the estrous cycle. *Peptides* **32** 1270–1275. (doi:10.1016/j.peptides.2011.03.022)

Primeaux SD, Blackmon C, Barnes MJ, Braymer HD & Bray GA 2008 Central administration of the RFamide peptides, QRFP-26 and QRFP-43, increases high fat food intake in rats. *Peptides* **29** 1994–2000. (doi:10.1016/j.peptides.2008.07.024)

Primeaux SD, Barnes MJ & Braymer HD 2013 Hypothalamic QRFP: regulation of food intake and fat selection. *Hormone and Metabolic Research* **45** 967–974. (doi:10.1055/s-0033-1353181)

Qaiser F, Wahab F, Wigar MA, Hashim R, Leprince J, Vaudry H, Tena-Sempere M & Shahab M 2012 Study of the role of novel RF-amide neuropeptides in affecting growth hormone secretion in a representative non-human primate (*Macaca mulatta*). *Endocrine* **42** 658–663. (doi:10.1007/s12020-012-9672-5)

Raffa RB 1988 The action of FMRFamide (Phe-Met-Arg-Phe-NH2) and related peptides on mammals. *Peptides* **9** 915–922. (doi:10.1016/0196-9781(88)90141-6)

Ramanjaneya M, Karteris E, Chen J, Rucinski M, Ziolkowska A, Ahmed N, Kagerer S, Jöhren O, Lehnert H, Malendowicz LK *et al.* 2013 QRFP induces aldosterone production via PKC and T-type calcium channel-mediated pathways in human adrenocortical cells: evidence for a novel role of GPR103. *American Journal of Physiology. Endocrinology and Metabolism* **305** E1049–E1058. (doi:10.1152/ajpendo.00191.2013)

Rastogi RK, D'Aniello B, Pinelli C, Fiorentino M, Di Fiore MM, Di Meglio M & Iela L 2001 FMRFamide in the amphibian brain: a comprehensive survey. *Microscopy Research and Technique* **54** 158–172. (doi:10.1002/jemt.1130)

Roch GJ, Busby ER & Sherwood NM 2011 Evolution of GnRH: diving deeper. *General and Comparative Endocrinology* **171** 1–16. (doi:10.1016/j.ygcen.2010.12.014)

Sherwood NM, Tello JA & Roch GJ 2006 Neuroendocrinology of protochordates: insights from *Ciona* genomics. *Comparative Biochemistry and Physiology A* **144** 254–271. (doi:10.1016/j.cbpa.2005.11.013)

Takayasu S, Sakurai T, Iwasaki S, Teranishi H, Yamanaka A, Williams SC, Iguchi H, Kawasawa YI, Ikeda Y, Sakakibara I *et al.* 2006 A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. *PNAS* **103** 7438–7443. (doi:10.1073/pnas.0602371103)

Tobari Y, Iijima N, Tsunekawa K, Osugi T, Haraguchi S, Ubuka T, Ukena K, Okanoya K, Tsutsui K & Ozawa H 2011 Identification, localisation and functional implication of 26RFa orthologue peptide in the brain of zebra finch (*Taeniopygia guttata*). *Journal of Neuroendocrinology* **23** 791–803. (doi:10.1111/j.1365-2826.2011.02179.x)

Tsutsui K 2009 A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): biosynthesis, mode of action and functional significance. *Progress in Neurobiology* **88** 76–88. (doi:10.1016/j.pneurobio.2009.02.003)

Tsutsui K & Ukena K 2006 Hypothalamic LPXRF-amide peptides in vertebrates: identification, localization and hypophysiotropic activity. *Peptides* **27** 1121–1129. (doi:10.1016/j.peptides.2005.06.036)

Tsutsui K, Bentley GE, Bedecarrats G, Osugi T, Ubuka T & Kriegsfeld LJ 2010*a* Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. *Frontiers in Neuroendocrinology* **31** 284–295. (doi:10.1016/j.yfrne.2010.03.001)

Tsutsui K, Bentley GE, Kriegsfeld LJ, Osugi T, Seong JY & Vaudry H 2010*b* Discovery and evolutionary history of gonadotrophin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. *Journal of Neuroendocrinology* **22** 716–727. (doi:10.1111/j.1365-2826.2010.02018.x)

Ukena K & Tsutsui K 2005 A new member of the hypothalamic RF-amide peptide family, LPXRF-amide peptides: structure, localization, and function. *Mass Spectrometry Reviews* **24** 469–486. (doi:10.1002/mas.20031)

Ukena K, Tachibana T, Iwakoshi-Ukena E, Saito Y, Minakata H, Kawaguchi R, Osugi T, Tobari Y, Leprince J, Vaudry H *et al.* 2010 Identification, localization, and function of a novel avian hypothalamic neuropeptide, 26RFa, and its cognate receptor, Gprotein-coupled receptor-103. *Endocrinology* **151** 2255–2264. (doi:10.1210/en.2009-1478)

Ukena K, Vaudry H, Leprince J & Tsutsui K 2011 Molecular evolution and functional characterization of the orexigenic peptide 26RFa and its receptor in vertebrates. *Cell and Tissue Research* **343** 475–481. (doi:10.1007/s00441-010-1116-z) (doi:10.1007/s00441-010-1116-z)

Vallarino M, Salsotto-Cattaneo MT, Feuilloley M & Vaudry H 1991 Distribution of FMRFamide-like immunoreactivity in the brain of the elasmobranch fish *Scyliorhinus canicula*. *Peptides* **12** 1321–1328. (doi:10.1016/0196-9781(91)90214-A)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd

Vallarino M, Feuilloley M, D'Aniello B, Rastogi RK & Vaudry H 1994 Distribution of FMRFamide-like immunoreactivity in the brain of the lizard *Podarcis sicula*. *Peptides* **15** 1057–1065. (doi:10.1016/0196-9781(94)90071-X)

Vallarino M, Feuilloley M, Thoumas JL, Demorgny R, Masini MA & Vaudry H 1995 Distribution of FMRFamide-like immunoreactivity in the brain of the lungfish *Protopterus annectens*. *Peptides* **16** 1187–1196. (doi:10.1016/0196-9781(95)02002-E)

Wahab F, Salahuddin H, Anees M, Leprince J, Vaudry H, Tena-Sempere M & Shahab M 2012 Study of the effect of 26RFand 43RF-amides on testosterone and prolactin secretion in the adult male rhesus monkey (*Macaca mulatta*). *Peptides* **36** 23–28. (doi:10.1016/j.peptides.2012.04.009)

Walker RJ, Papaioannou S & Holden-Dye L 2009 A review of FMRFamide- and RFamide-like peptides in metazoa. *Invertebrate Neuroscience* **9** 111–153. (doi:10.1007/s10158-010-0097-7)

Yamamoto T, Wada T & Miyazaki R 2008 Analgesic effects of intrathecally administered 26RFa, an intrinsic agonist for GPR103, on formalin test and carrageenan test in rats. *Neuroscience* **157** 214–222. (doi:10.1016/j.neuroscience.2008.08.061)

Received in final form 8 January 2014
Accepted 6 February 2014
Accepted Preprint published online 14 February 2014

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-13-0207

Published by Bioscientifica Ltd