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ORIGINAL ARTICLE

Study of the role of novel RF-amide neuropeptides in affecting growth hormone secretion in a representative non-human primate (*Macaca mulatta*)

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Abstract RF amide peptide family with distinctive terminal –Arg–Phe–NH_2 signature is evolutionarily conserved from invertebrates to mammals. These neuropeptides have been shown to affect diverse functions in invertebrates and vertebrates including influencing pituitary hormone secretion. More recently, two members of this family 26-amino acid and 43-amino acid RF amide peptide (26RFa and 43RFa, respectively) originally isolated from frog have been cloned in rats and humans. Actions of these peptides on hormone secretion have not been studied in primates. In the present study, effect of iv administration of three different doses of human 26RFa and 43RFa on GH secretion was studied in a representative higher primate, the rhesus monkey. As control against these two peptides, normal saline and a scrambled sequence of 26RFa was administered. A set of four intact adult male monkeys received the administration in a random order. Peripheral blood samples were obtained from the chairrestrained but fully conscious animals for a period of 30 min before and 240 min after the administration at 15-min intervals. For quantitative measurement of GH concentration, a human GH chemiluminescent immunometric assay was used. Peripheral administration of 38 and 76 nmol doses of 26RFa significantly ($P < 0.05$) stimulated GH AUC during a 0–120 min period after injection of 26RFa. In contrast to 26RFa, administration of 43RFa appeared to suppress GH levels during the later stages of the sampling i.e. from 120 to 240 min period. Mean AUC during the period was significantly ($P < 0.05$) reduced by 76 nmol dose of 43RFa, while 38 nmol dose of 43RFa also had similar effect but lacked full statistical significance ($P = 0.058$). To our knowledge present study reports for the first time-specific stimulatory effect of 26RFa on the GH secretion and a novel inhibitory and delayed effect of 43RFa on the GH secretion in higher primates. In conclusion, present findings extend evidence for endocrine actions of RF amides in primates and suggest differential effect of these peptides on GH secretion in primates.

Keywords Growth hormone · 26RF amide · 43RF amide · Rhesus monkey

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Introduction

Almost 30 years ago, tetrapeptide FMRF amide was isolated from the ganglia of the venus clam, *Macrocallista nimbosa* [1]. Since then, a good number of peptides possessing the Arg–Phe–NH_2 motif at their C-terminal have been identified in all the groups of invertebrates [for review, [2]]. These peptides now classified as members of a large family, are collectively termed the RF amide (RFa) peptides. Over the last decade or so, vertebrate brain has also been shown to produce number of RFa neuropeptides

with vast variety of functions. To date, many important physiological roles in neuroendocrine, behavioral, sensory, and autonomic functions have been defined for these RFa peptides [3–5].

A 26-amino acid RFa neuropeptide, originally isolated from the frog and named 26RFa, has been cloned in human and rat [6]. This peptide, also known as P518 [7] and QRFP [8] is the ligand for the previously orphan G-protein-coupled receptor (GPR) 103. Through bioinformatics approaches, precursor gene for this novel RFa peptide was identified [7, 8]. Precursor gene, encodes preproprotein that can be processed into several potential peptides, including 26RFa and a 43RFa peptide (also termed QRFP) [7, 8]. Both of these peptides activate GPR103, but 43RFa exhibits more potent agonistic activity. A combination of in vitro and in vivo approaches applied in rodents showed that 26RFa and 43RFa have effects on hormone secretion and behavior. Icv and systemic administration of 26RFa was able to elicit gonadotropin release only in female rats [9]. Centrally injected 26RFa also exhibited orexigenic activity in mice [6]. Intravenous administration of 43RFa in rats was observed to stimulate aldosterone release [7]. While central administration of 43RFa in mice induced feeding behavior, intensified grooming response, influenced blood pressure accompanied by increased general locomotor activity and metabolic rate [10]. Studies using the antagonist against the Y1 and RFa receptor activity have shown that RFa receptor may also play a role in regulation of cardiovascular activity by affecting the food intake and energy homeostasis through interaction with the central circuits [11]. The prolactin-releasing peptide (PrRP), another peptide member of this family has been shown to have potency comparable to TRH in effecting prolactin release. In vitro and in vivo studies in rats have shown that PrRP contributes in modulating circulatory prolactin levels along with TRH. In vitro studies using the rat pituitary primary cell cultures showed that the prolactin responses to TRH was clearly augmented by PrRP (10–30 fold increase) and in vivo the response was increased up to three-folds [12].

Keeping in view the established fact that these RFa are also present in the higher primates including humans but with a very little knowledge of their endocrine functions' present study was designed to determine the effect of recently characterized two human RFa, 26RFa, and 43RFa on GH secretion in the adult male rhesus monkey which is a representative higher primate model.

Materials and methods

Animals

A set of four chair-restrained adult (7–8 years) intact male rhesus monkeys (*Macaca mulatta*) (Body weight: 6.0–8.0 kg) was used. Individual cages were used to house the animals under semi controlled colony conditions in the Department's primate facility. The animals were fed monkey diet at 1,300–1,330 h daily with fresh fruits supplements in the morning (0900–0930 h). Water was available ad libitum. The animals were gradually habituated to chair restraint 8 weeks before commencing of the experiments. Each animal was restrained on chair for a maximum period of 4 h daily. Animals were also trained to take their food and water while chair restrained. For the ease of fixing or removing from the restraining chair, animals were sedated by intramuscular administration of ketamine hydrochloride (3–5 mg/kg BW). However, sampling started only after animals had regained full consciousness. All animal studies were approved by the Departmental Committee for Care and Use of Laboratory Animals.

Blood sampling

Serial blood samples (2.5 ml) were taken in hepranized syringes attached to an indwelling cannula placed in the saphanous vein an hour before sampling under ketamine sedation (5 mg/kg BW, im). After each sample approximately 3 ml of normal saline containing 5 IU of heparin/ml was injected to compensate the lost blood volume and to preserve patency of the cannula. The samples were immediately transferred to culture tubes kept on ice. After completion of sampling, tubes were centrifuged at 3,000 rpm at 4 °C and plasma was extracted and stored at −15 °C until assayed.

Reagents

Heparin (Rotexmedica, Trittau, Germany) and ketamine hydrochloride (Ketler, Astarapin, Germany) were purchased locally. Human RFa (26RFa, 43RFa, and Sc 26RFa) were synthesized in the lab of the one of the co-authors (HV) and the details have been described before [9]. Working solutions of RFa were made in normal saline.

General experimental design

The experiment comprised of a random administration of different doses of RFa and controls on 8 non-consecutive days with a gap of 4 days. All four animals were used on each day of sampling. A total of 20 blood samples were taken from each animal on each day of sampling. Sampling duration was about 5 h (1100–1800 h). The samples were obtained at 15-min intervals for 30 min before injections

(−30, −15, and 0 min) and for 240 min thereafter. RF amides (19, 38, and 76 nmol/animal) or normal saline as vehicle were administered as iv bolus immediately after taking 0 min sample. A scrambled sequence of 26RFa was also used as a specific control to 26RFa. Volume of doses of the peptides and saline was 1 ml. Doses of the amides were estimated from neuroendocrine effective doses of kisspeptin a type of RF peptides, in monkeys [13, 14].

Hormone assays

The quantitative determination of GH concentration in monkey plasma was done by a Immulite/Immulite 1000 human GH chemiluminescent immunometric assay (Euro/DPC LTD Gwyneed, UK). All the assays were done according to the instructions given by the kit manufacturers. Analytical sensitivity of the kit was 0.026 m IU/l. Intra- and inter-assay % CV was 5.96 and 2.56 %, respectively.

Statistical analyses

All data presented are mean ± SEM. Paired Student's *t* test was employed to determine differences between pre- and post-treatment mean GH concentrations, and between mean area under the curve (AUC) of post-treatment GH values. One-way analysis of variance (ANOVA) with repeated measures followed by Dunnett's test was used to analyze time course of change in mean plasma GH concentrations before and after iv injection of various doses of RFa and saline. Statistical significance was set at $P \leq 0.05$.

Results

Time course of change in mean plasma GH after administration of 26RFa

Pattern of GH secretion before and after administration of 26RFa is shown in Fig. 1. Peripheral administration of 38 nmol of scrambled sequence of 26RFa (Sc 26RFa) appeared to occasion a progressive decrease in plasma GH levels. However, mean plasma GH concentration after the injection of Sc 26RFa showed a non significant decrease. Though administration of the 3 doses of 26RFa apparently increased GH levels within an hour, repeated measures ANOVA did not indicate difference between time points. The comparison between mean pre-treatment (−30–0 min) and mean post-treatment (15–120 min) plasma GH levels showed non-significant effect on GH levels after administration of Sc 26RFa and 19 nmol of 26RFa. Whereas both 38 and 76 nmol of 26 RFa induced a significant ($P < 0.05$) increase in post treatment GH levels.

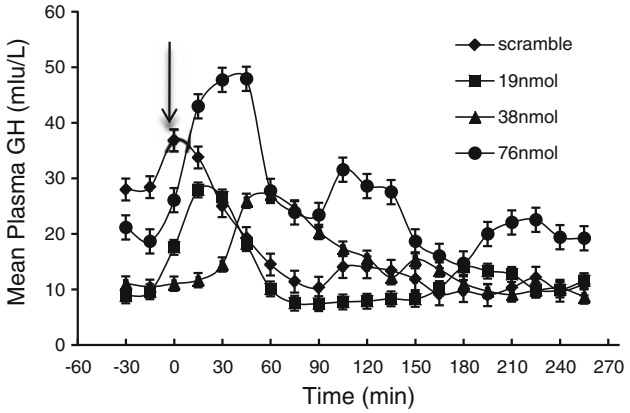

Fig. 1 Time course of change in mean (±SEM) plasma GH concentrations before and after iv bolus injection of 38 nmol of Sc 26RFa and 19, 38, and 76 nmol of 26RFa in adult male rhesus monkeys ($n = 4$). *Arrow* indicates time of administration of the injection. No significant difference was evident between mean GH at different time points

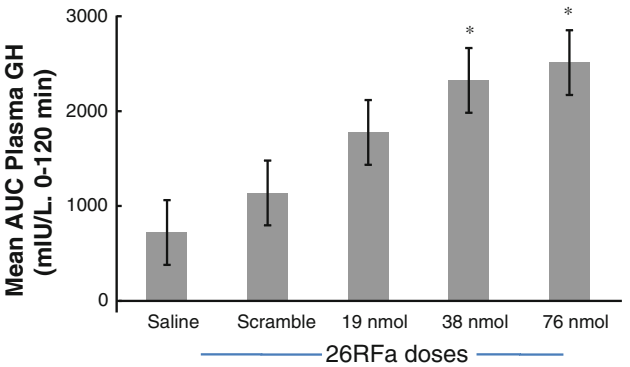

Fig. 2 Comparison between AUC of plasma GH concentrations (mean ± SEM) observed during 0–120 min period after injection of saline, Sc 26RFa and various doses of 26RFa, in adult male rhesus monkeys ($n = 4$) *$P < 0.05$ versus saline and Sc 26RFa

Comparison of mean AUC of plasma GH concentrations observed during post 26RFa injection periods

Comparison between AUC of plasma GH concentrations during a 0–120 min period after injection of saline, Sc 26RFa, and various doses of 26RFa, in adult male monkeys is shown in Fig. 2. Paired *t* test analyses showed that the mean AUC was significantly increased ($P < 0.05$) by the 38 and 76 nmol doses of 26RFa when compared to those caused by saline and Sc 26RFa.

Time course of change in mean plasma GH after administration of 43RFa

Saline administered peripherally as vehicle of 43RFa caused no affect on plasma GH concentrations in the

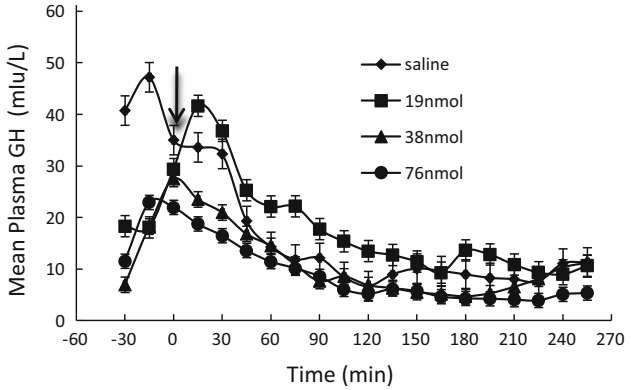

Fig. 3 Time course of change in mean (±SEM) plasma GH concentrations before and after iv injection of saline and 19, 38, and 76 nmol of 43RFa in adult male rhesus monkeys ($n = 4$). *Arrow* indicates time of administration of the injection. Repeated measures ANOVA followed by post hoc Dunnett's test showed that mean plasma GH was significantly reduced by 38 and 76 nmol doses of 43RFa at time periods 120–210 min as compared to the respective 0 min value

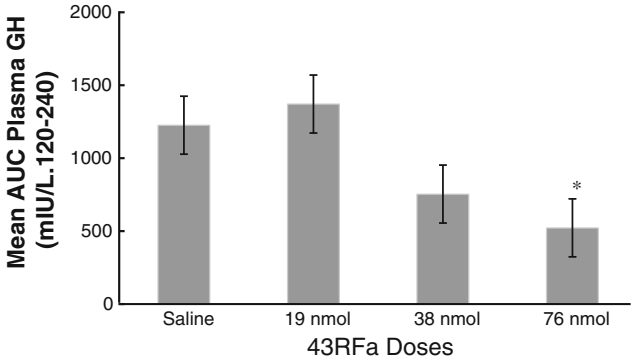

Fig. 4 Comparison of mean (±SEM) AUC of plasma GH concentrations during 120–240 min period after injection of saline and various doses of 43RFa in adult male rhesus monkeys ($n = 4$) *$P < 0.05$ versus saline. Comparison of saline and 38 nmol means revealed a difference which was approaching significance ($P = 0.058$)

observation period (Fig. 3). Analysis of changes, in mean plasma GH concentrations after iv bolus injection of different doses of 43RFa showed a slight and short lived rise and then a progressive decline. Repeated measures ANOVA showed a significant decrease in mean GH concentration after administration of 38 and 76 nmol of 43RFa at time points between 120 and 240 min as compared to 0 min value ($P < 0.05$). No significant effect on GH levels was observed at any time point after administration of 19 nmol 43RFa. The comparison between mean pre-treatment (−30–0 min) and mean post-treatment (15–120 min) plasma GH levels showed that there was no significant alteration observed after the administration of normal saline and any dose of 43RFa.

Comparison of mean AUC of plasma GH concentrations observed during post 43RFa injection periods

Comparison of mean AUC of plasma GH concentrations after injection of saline and various doses of 43RFa during the period 120–240 min in adult male rhesus monkeys is shown in Fig. 4. Paired t-test analyses showed that GH AUC was significantly ($P < 0.05$) suppressed by 76 nmol dose of 43RFa as compared to saline. Administration of 38 nmol dose of 43RFa also almost significantly ($P = 0.058$) decreased the AUC, while 19 nmol dose of the peptide did not affect the AUC. Comparisons of GH AUC observed during an earlier period (0–120 min) after administration of saline and different doses of 43RFa revealed no stimulatory or inhibitory effects of 43RFa (data not shown).

Discussion

The main finding of the present study was that iv administration of 26RFa appeared to acutely increase plasma GH levels in adult male monkeys. Comparison of post-treatment GH levels with their respective pre-treatment values showed that 38 and 76 nmol doses of 26RFa stimulated the GH release. That the increase in GH levels was specific to the amide treatment, was evident by a lack of any such effect after the administration of saline and Sc 26RFa. Present study, therefore reports for the first time specific stimulatory effect of 26RFa on the GH secretion in a higher primate. Mean post-treatment GH concentrations appeared to rise with dose of 26RFa when compared to saline, but there was not much difference between effects of 38 and 76 nmol doses. The observed stimulatory effect of 26RFa on GH secretion in adult male rhesus monkeys is in line with the previous findings in some other vertebrates. LPXRF amide peptides, gfLPXRFa-1, -2, and -3, have been observed to stimulate GH release in a dose-dependent manner from cultured pituitary cells of sockeye salmon. Moreover, gfLPXRFa-1, -2, and -3 also stimulated release of FSH and LH [15]. In vitro experiments in amphibians also support our results, as fGRP a type of RF amide peptides stimulated GH release from cultured frog pituitary cells, in a dose related manner [16]. It was also demonstrated that in juvenile and adult frogs exogenously administered RFa peptide elevated GH levels in blood [14]. Our results are also similar to the observations made in rats where icv injections of RFRP-3 another RFa profoundly increased plasma GH levels [17]. However, in ovine pituitary cells RFRP-3 treatment was shown not altering GH mRNA [18]. Parenthetically, kisspeptin RFa's have been recently shown to increase release of GH from primary

cultures of female baboon pituitaries in a dose dependant manner [19]. Taken together, foregoing evidence suggests that GH stimulating actions of RFa peptides prevalent in lower vertebrates are also present in primates.

In contrast to 26RFa, iv administration of 43RFa appeared to suppress GH levels during the later stages of the sampling, i.e., from 120 to 240 min period. Owing to nature of the effect of 43RFa, statistical comparisons were extended to include the terminal epoch of the sampling period as well. Parenthetically, analysis of plasma GH AUC during the earlier period (0–120 min) did not reveal any stimulatory or inhibitory effects of any dose of 43RFa. Analysis of time course of change showed that decrease in GH levels was specific to the amide treatment as saline treatment did not significantly affect GH levels. Present study, therefore, reports for the first time a novel inhibitory and delayed effect of 43RFa on the GH secretion in higher primates. Dose dependency trend was evident with this action of 43RFa as mean post-treatment GH AUC appeared to decrease, with increase in the dose. However, the mean AUC was significantly suppressed only by the highest dose of 43RFa when compared to saline.

Our observation of peripheral action of 43RFa to reduce the plasma GH levels in adult male rhesus monkeys, however, has limited support from previous findings. Two novel RFa peptides in sea lamprey, the 25 aa peptide RFa-A and the 20 aa peptide RFa-B have been observed to inhibit the GH secretion [20]. Synthetic RFa-A and -B inhibited GH mRNA expression in a concentration-dependent fashion in vitro [20]. Our results are also in line with the findings in teleosts where GH secretion from the pituitary was inhibited by salmon PrRP in vivo, but no change in GH release was observed in vitro [21, 22]. In rats, icv administration of PrRP inhibited GH release from the pituitary [23, 24]. The inhibitory effects of PrRP on GH were diminished by depletion or neutralization of somatostatin [23] indicating that inhibitory effect of PrRP on GH was mediated via somatostatin release. In favor of this notion, it has been shown that in rat's axon terminals of PrRP appeared to contact among others, somatostatin immunoreactive neuronal elements in the arcuate nucleus [25–27]. Together, these results suggest that 43RFa peptides can potentially inhibit GH secretion both indirectly through the hypothalamic action as well as by a direct pituitary action. However, further investigation is necessary to elucidate the mechanism underlying the suppressive effects of 43RF amide peptides on GH secretion in monkeys as observed in the present study.

There can be two possible reasons for the observed opposite effects of 26RFa and 43RFa on GH secretion in monkeys. One possible reason for this dual effect can be that the two amides utilize two different receptors. Previous findings support that apart from GPR103 there may exist other binding sites for 26RFa. In the brain and spinal cord of rats, 26RFa binding sites were widely distributed, whereas the expression of GPR103 mRNA was more discrete, notably in the midbrain, the pons, and the medulla oblongata, suggesting that 26RFa can bind to a receptor(s) other than GPR103. Competition experiments confirmed that 26RFa interacts with receptor distinct from GPR103 that may be NPFF2 [28]. Functional assays and binding studies indicated that binding of 43RFa to GPR103 has the same efficacy as that of 26RFa. However, 43RFa has been shown to be slightly more potent than 26RFa with respect to cAMP inhibition [8]. Second, the expression pattern of the peptides to their cognate receptors may have selectivity for GHRH or SRIF systems in the hypothalamus. Therefore, selective presence of these amides and their receptors in regions of the hypothalamus that entrain inhibitory and excitatory drives to GH release from pituitary, and likelihood of multiple receptors utilized by these two peptides, provide the possible reasons for the dual effect of 26RFa and 43RFa on GH release.

Taken together, all the foregoing studies support the view that the large family of RF amide peptides contributes to the multifactorial regulation of pituitary hormone particularly GH release in vertebrates and our results advance the understanding of such RF amide peptide actions in primates. Design of the current study did not allow us to infer much whether actions of 26RFa or 43RFa were pharmacological or physiological in nature. Parenthetically, previous studies point toward the physiological role of RFa in the regulation of the GH secretion in vertebrates. gfLPXRFa-1, -2, and -3, stimulated the GH release in a dose-dependent manner from cultured pituitary cells of sockeye salmon, this stimulatory effect may be taken as physiological action, because threshold concentrations ranged from less than 10^{-5} M [13]. Similar findings were observed in amphibians, where RFa fGRP peptide stimulated GH release from cultured frog pituitary cells, in a dose-related manner with threshold concentration ranging between 10^{-9} and 10^{-8} M suggesting a physiological action [9]. In conclusion, present findings demonstrate that 26RFa and 43RFa differentially effect GH release in the rhesus macaque. However, further experiments are required to asses if these peptides are involved in physiological regulation of GH secretion in higher primates.

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Conflict of interest None of the authors have any conflict of interest with respect to this study.

References

1. D.A. Price, M. Greenberg, Structure of a molluscan cardioexcitatory neuropeptide. Science **197**, 670–671 (1977)
2. N. Chartrel, C. Dujardin, J. Leprince, L. Desrues, M.C. Tonon, E. Cellier, P. Cosette, T. Jouenne, G. Simonnet, H. Vaudry, Isolation, characterization, and distribution of a novel neuropeptide, Rana RFamide (R-RFa), in the brain of the European green frog *Rana esculenta*. J. Comp. Neurol. **448**, 111–127 (2002)
3. Y. Ibata, N. Iijima, Y. Kataoka, K. Kakihara, M. Tanaka, M. Hosoya, S. Hinuma, Morphological survey of prolactin-releasing peptide and its receptor with special reference to their functional roles in the brain. Neurosci. Res. **3**, 223–230 (2000)
4. P. Panula, A.A. Aarnisalo, K. Wasowicz, Neuropeptide FF, a mammalian neuropeptide with multiple functions. Prog. Neurobiol. **48**, 461–487 (1996)
5. P. Panula, E. Kalso, M. Nieminen, V.K. Kontinen, A. Brandt, A. Pertovaara, Neuropeptide FF and modulation of pain. Brain Res. **848**, 191–196 (1999)
6. N. Chartrel, C. Dujardin, Y. Anouar, J. Leprince, A. Decker, S. Clerens S, J.C. Do-Re′go, F. Vandesande, C. Llorens-Cortes, J. Costentin, J.C. Beauvillain, H. Vaudry, Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. Proc. Natl. Acad. Sci. USA. **100**, 247–252 (2003)
7. Y. Jiang, L. Luo, E.L. Gustafson, D. Yadav, M. Laverty, N. Murgolo, G. Vassileva, M. Zeng, T.M. Laz, J. Behan, P. Qiu, L. Wang, S. Wang, M. Bayne, J. Greene, F. Monsma Jr., F.L. Zhang, Identification and characterization of a novel RF-amide peptide ligand for orphan G-protein-coupled receptor SP9155. J. Biol. Chem. **278**, 652–657 (2003)
8. S. Fukusumi, H. Yoshida, R. Fujii, M. Maruyama, H. Komatsu, Y. Habata, Y. Shintani, S. Hinuma, M. Fujino, A new peptidic ligand and its receptor regulating adrenal function in rats. J. Biol. Chem. **278**, 387–395 (2003)
9. V.M. Navarro, F.R. Fern′andez1, R. Nogueiras, E. Vigo1, S. Tovar, N. Chartrel, O. Le Marec, J. Leprince, E. Aguilar1, L. Pinilla1, C. Dieguez, H. Vaudry, M. Tena-Sempere, Novel role of 26RFa, a hypothalamic RFamide orexigenic peptide, as putative regulator of the gonadotropic axis. J. Physiol. 573.**1**, 237–249 (2006)
10. S. Takayasu, T. Sakurai, S. Iwasaki, H. Teranishi, A. Yamanaka, S.C. Williams, H. Iguchi, Y.I. Kawasawa, Y. Ikeda, I. Sakakibara, K. Ohno, R.X. Ioka, S. Murakami, N. Dohmae, J. Xie, T. Suda, T. Motoike, T. Ohuchi, M. Yanagisawa, J. Sakai, A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. Proc. Natl. Acad. Sci. USA **130**, 7438–7443 (2006)
11. K. Gruber, W. Fan, H. Akerberg, D. Larhammar, M. Chee, W. Colmers, R. Cone, Neuropeptide Y and gamma-melanocyte stimulating hormone (γ-MSH) share a common pressor mechanism of action. Endocrine **35**(3), 312–324 (2009)
12. C. Spuch, Y. Diz-Chaves, D. Pérez-Tilve, M. Alvarez-Crespo, F. Mallo, Prolactin-releasing peptide (PrRP) increases prolactin responses to TRH in vitro and in vivo. Endocrine **31**(2), 119–124 (2007)
13. M. Shahab, C. Mastronardi, S.B. Seminara, W.F. Crowley, S.R. Ojeda, T.M. Plant, Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. Proc Natl. Acad. Sci. USA **102**(6), 2129–2134 (2005)
14. T.M. Plant, S. Ramaswamy, M.J. Dipietro, Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges. Endocrinology **147**(2), 1007–1013 (2006)
15. M. Amano, S. Shunsuke Moriyama, M. Iigo, S. Kitamura, N. Amiya, K. Yamamori, K. Kazuyoshi Ukena, K. Tsutsui, Novel fish hypothalamic neuropeptides stimulate the release of gonadotrophins and growth hormone from the pituitary of sockeye salmon. J. Endocrinol. **188**, 417–423 (2006)
16. A. Koda, K. Ukena, H. Teranishi, S. Ohta, K. Yamamoto, S. Kikuyama, K. Tsutsui, A novel amphibian hypothalamic neuropeptide: isolation, localization, and biological activity. Endocrinology **143**, 411–419 (2002)
17. A.M. Johnson, K. Tsutsu, S.G. Fraley, Rat RFamide related peptide-3 stimulates GH secretion, inhibits LH secretion, and has variable effects on sex behavior in the adult male rat. Horm. Behav. **51**, 171–180 (2007)
18. I.P. Sari, A. Rao, J.T. Smith, A.J. Tilbrook, I.J. Clark, Effect of RF-amide-related peptide-3 on Luteinizing hormone and follicle-stimulating hormone synthesis and secretion in ovine pituitary gonadotropes. Endocrinology, **15**, 5549–5556 (2009)
19. R.M. Luque, J. Córdoba-Chacón, M.D. Gahete, V.M. Navarro, M. Tena-Sempere, R.D. Kineman, J.P. Castaño, Kisspeptin regulates gonadotroph and somatotroph function in nonhuman primate pituitary via common and distinct signaling mechanisms. Endocrinology **152**(3), 957–966 (2011)
20. S. Moriyama, M. Kasahara, N. Amiya, A. Takahashi, M. Amano, A. Stacia, S.A. Sower, K. Yamamori, H. Kawauchi, RFamide peptides inhibit the expression of melanotropin and growth hormone genes in the pituitary of an Agnathan, the sea lamprey, *Petromyzon marinus*. Endocrinology **148**, 3740–3749 (2007)
21. S. Moriyama, T. Ito, A. Takahashi, M. Amano, S.A. Sower, T. Hirano, K. Yamamori, H. Kawauchi, A homolog of mammalian PRL-releasing peptide (fish arginyl-phenylalanyl-amide peptide) is a major hypothalamic peptide of PRL release in teleost fish. Endocrinology **143**, 2071–2079 (2002)
22. T. Sakamoto, M. Fujimoto, M. Andot, Fishy tales of prolactin-releasing peptide. Int. Rev. Cytol. **225**, 91–130 (2003)
23. N. Iijima, Y. Matsumoto, T. Yano, M. Tanaka, T. Yamamoto, K. Kakihara, Y. Kataoka, Y. Tamada, H. Matsumoto, N. Suzuki, S. Hinuma, Y. Ibata, A novel function of prolactin-releasing peptide in the control of growth hormone via secretion of somatostatin from the hypothalamus. Endocrinology **142**, 3239–3243 (2001)
24. S.Q. Zhang, S. Inoue, M. Kimura, Sleep-promoting activity of prolactin releasing peptide (PrRP) in the rat. NeuroReport **12**, 3173–3176 (2001)
25. M.M. Taylor, W.K. Samson, The prolactin releasing peptides: RF-amide peptides. Cell. Mol. Life Sci. **58**, 1206–1215 (2001)
26. W.K. Samson, M.M. Taylor, J.R. Baker, Prolactin-releasing peptides. Regul. Pept. **114**, 1–5 (2003)
27. B. Sun, K. Fujiwara, S. Adachi, K. Inoue, Physiological roles of prolactin releasing peptide. Regul. Pept. **126**, 27–33 (2005)
28. F. Bruzzone, B. Lectez, D. Alexandre, S. Jégou, L. Mounien, H. Tollemer, D. Chatenet, J. Leprince, M. Vallarino, H. Vaudry, N. Chartrel, Distribution of 26RFa binding sites and GPR103 mRNA in the central nervous system of the rat. J. Comp. Neurol. **503**, 573–591 (2007)