Current Trends in Endocrinology
Vol. 7, 2014

Review

The role of RFamide-related peptide-3 (RFRP-3) in the regulation of the reproductive function: Versatile effects and new perspectives

Caroline Ancel and Valérie Simonneaux*

Institut des Neurosciences Cellulaires et Intégratives, CNRS-UPR 3212, Université de Strasbourg, 5 rue Blaise Pascal, 67084 Strasbourg, France

ABSTRACT

The activity of the hypothalamo-pituitary-gonadal axis involves the release of gonadotrophin-releasing hormone (GnRH) into the portal blood system, subsequently stimulating the release of gonadotrophins, luteinising hormone (LH) and follicle-stimulating hormone (FSH). In turn, gonadotrophins act on the gonads to regulate reproductive activity. Characterisation of GnRH neuron regulators has significantly progressed in recent years. In 2000 the *RFamide-related peptide* (*rfrp*) gene was identified in humans and shown to produce two peptides, RFRP-1 and RFRP-3. *Rfrp* is the mammalian ortholog of avian *gonadotrophin-inhibitory hormone* (*gnih*), which was shown to inhibit gonadotrophin release from quail pituitaries. This finding spurred great interest in the roles of RFRP peptides, and especially RFRP-3, in the regulation of endocrine functions in mammals. A large number of studies have since then aimed at determining the involvement of RFRP-3 in the regulation of the reproductive function and suggest that the effect of the peptide on the gonadotrophic axis may vary according to species, gender and physiological states. This review comprehensively summarises the variable effects of RFRP-3 that are observed on the mammalian reproductive function.

KEYWORDS: RFamide-related peptide-3 (RFRP-3), gonadotrophin-inhibitory hormone (GnIH), gonadotrophin-releasing hormone (GnRH), reproduction, hypothalamus, neuroendocrinology

1. INTRODUCTION

The gonadotrophin-releasing hormone (GnRH) neurons in the rostral hypothalamus (preoptic area and organum vasculosum of lamina terminalis) represent the final common pathway in the neural regulation of the hypothalamo-pituitary-gonadal (HPG) axis. These neurons release GnRH into the portal blood system, inducing the downstream secretion of gonadotrophins luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. Finally, LH and FSH regulate the production of sex steroids by the gonads, which will in turn feedback at various levels of the gonadotrophic axis. GnRH neurons are the target of various neurotransmitters, neuropeptides, and peripheral hormones known to modulate their function in order to fine-tune the activity of the HPG axis with environmental, metabolic and endocrine signals.

In recent years, the characterisation of GnRH neuron activity regulators has significantly progressed,

*Corresponding author: Valérie Simonneaux, Institut des Neurosciences Cellulaires et Intégratives, UPR CNRS 3212, Université de Strasbourg, Département de Neurobiologie des Rythmes, 5 rue Blaise Pascal, 67084 Strasbourg, France. simonneaux@inci-cnrs.unistra.fr

Work supported by the French "Agence Nationale de la Recherche" (grant n° BLAN-07-0056), the NOVO Nordisk Foundation, and the Danish Medical Research Council.

notably with the discovery of kisspeptins (Kp). In 2003, two studies concurrently indicating that the Kp receptor (Kiss1R), and therefore its ligands, were essential for normal reproduction [1, 2] prompted intensive research on the involvement of Kp in the regulation of the HPG axis. The presence of Kiss1R in GnRH neurons [3, 4] and the fact that Kp fibres come into close apposition to GnRH cell bodies in the preoptic area and fibres in the median eminence [5] suggest that Kp could be acting directly at the level of GnRH neuron cell bodies and via GnRH nerve terminals in the median eminence [6]. It has now been thoroughly demonstrated that Kp are potent stimulators of HPG axis activity and that they are central gatekeepers of key aspects of reproductive function; see [7] for review.

Although it is now apparent that Kp are central players in the regulation of GnRH neuron activity, other modulators of HPG axis activity have been identified. Notably, novel peptides of the RFamide family of peptides of which Kp is also a member, which share a common C-terminal LPXRFamide (X = L or Q) motif, have been identified in mammals. RFamide-related peptide-1 and -3 (RFRP-1 and RFRP-3) were isolated in mammals in 2000 [8] and since then a large number of studies have sought to identify the role of these peptides in the regulation of endocrine functions.

2. Discovery, localisation and sites of action of rfrp-3

2.1. Discovery and evolutionary history

RFRP-3 is part of the large family of RFamide peptides, which share a common Arg-Phe-NH_2 motif at their C-terminus. The first RFamide peptide was discovered in the clam *Macrocallista nimbosa* and reported to exert cardioexcitatory effects [9]. Since then, other RFamide peptides have been isolated from invertebrates [10] and the first report of an RFamide peptide in a vertebrate was done some 30 years ago; LPLRFamide was isolated from chicken brain [11] and shown to have vasopressor and stimulatory effects on neurons in mammals [12, 13]. Since then at least five different genes encoding RFamide peptides have been identified in mammals: PrRP, NPFF, QRFP/ 26RFa, Kp and RFRP [8, 14-23]. One of these genes, *RFamide-related peptide* (*rfrp*), was identified in mammals in 2000 [8] concurrently with the discovery of its avian ortholog, *gonadotrophin-inhibitory hormone* (*gnih*) [24]. The *rfrp* gene encodes a precursor which produces two peptides of various sizes in mammals: RFRP-1 and RFRP-3 (Table 1) [8]. As GnIH was shown to inhibit gonadotrophin release from cultured quail pituitaries [24], the involvement of RFRP-1 and RFRP-3 in the regulation of neuroendocrine functions in mammals was examined. As an initial study in rats showed that RFRP-1 had no effect on gonadotrophin secretion [8], studies have aimed at investigating the involvement of RFRP-3 in the regulation of mammalian reproduction. However, recent evidence indicates that the effect of RFRP-1 on the gonadotrophic axis could be species-dependent [25, 26], and therefore the relative role of this peptide in the regulation of the reproductive function deserves further investigation. Nevertheless, accumulating evidence now indicates that RFRP-3 is involved in the regulation of the hypothalamo-pituitary-gonadal axis in mammals, and this will be addressed in detail in this review.

2.2. Localisation of RFRP neurons in the mammalian brain

Both *in situ* hybridization studies and immunohistochemical mapping experiments have been carried out to localise RFRP-expressing cells. However it is important to bear in mind that the immunohistochemical findings could be affected by a possible variation in specificity of the antibodies used. Indeed, a variety of antibodies has been characterized for the study of RFRP-immunoreactivity (-ir), including a polyclonal antibody raised against avian GnIH [24], an antiserum against the rat RFRP precursor peptide [27], a white crowned sparrow GnIH antiserum [28, 29] and an antibody raised in guinea pigs against human RFRP-3 [30]. Moreover, because of the differences in RFRP-like sequences among mammalian species (Table 1), a given antibody could result in variable labeling from one species to another.

In the mouse brain, RFRP-ir cells have been localised in the diencephalon, pons, medulla and dorsomedial nucleus of the hypothalamus (DMH) [31]. In rats, RFRP-ir cells are located mainly in the DMH and in regions surrounding the ventromedial nucleus and tuberomammillary

Table 1. Alignment of amino acid sequences of LPXRFa (X = L or Q) peptides in mammals and the quail.

MPHSFAN**LPLRFa**	Human RFRP-1	[87]
VPN**LPQRFa**	Human RFRP-3	[87]
SGRNMEVSLVRQVLN**LPQRFa**	Monkey RFRP-3	[87]
SLTFEEVKDWAPKIKMNKPVVNKMPPSAAN**LPLRFa**	Bovine RFRP-1	[88]
AMAHLPLRLGKNREDSLSRWVPN**LPQRFa**	Bovine RFRP-3	[89]
SVTFQELKDWGAKKDIKMSPAPANKVPHSAAN**LPLRFa**	Rat RFRP-1*	[8]
ANMEAGTMSHFPS**LPQRFa**	Rat RFRP-3	[90]
SPAPANKVPHSAAN**LPLRFa**	Siberian hamster RFRP-1	[57]
TLSRVPS**LPQRFa**	Siberian hamster RFRP-3	[57]
SPAPANKVPHSAAN**LPLRFa**	Syrian hamster RFRP-1*	[29]
ILSRVPS**LPQRFa**	Syrian hamster RFRP-3*	[29]
SIKPSAY**LPLRFa**	Quail GnIH	[24]
SLNFEEMKDWGSKNFMKVNTPTVNKVPNSVAN**LPLRFa**	Quail GnIH-RP-1*	[91]
SSIQSLLN**LPQRFa**	Quail GnIH-RP-2	[91]

*Putative LPXRFa peptides hypothesized from their precursor mRNA sequences.

nucleus [27, 32]. In rats, *rfrp* mRNA has been detected in cells located in the DMH and dorsomedial parts of the ventromedial nucleus with cells extending rostral to the anterior hypothalamus and the ventral perifornical area [33]. In another study, RFRP-ir and mRNA were detected in the DMH in Syrian hamsters, mice and rats [29, 34]. In Siberian hamsters, RFRP-ir cell bodies are distributed in the medial region of the hypothalamus spanning from the anterior hypothalamic area, DMH, and premammillary nucleus [26]. In sheep, *in situ* hybridisation has shown RFRP-expressing cells in the ventral region of the paraventricular nucleus and DMH [28, 35, 36]. A similar distribution was described using immunohistochemistry [28, 30].

Although inter-species differences appear in the distribution of RFRP neurons, possibly due to antibody specificity issues, the mediobasal hypothalamus, particularly the DMH appears as a key region containing these neurons. This is of interest as the DMH has been implicated in a variety of behavioral and physiological responses, including those associated with feeding, reproduction, stress, circadian rhythms, and thermogenesis. Moreover, the DMH receives inputs from a large number of hypothalamic regions, suggesting that neurons in this area could integrate environmental and physiological signals to regulate endocrine responses.

2.3. Sites of action of RFRP-3 in mammals

In various mammalian species including humans, RFRP fibre networks are found in multiple brain regions including the preoptic area, the arcuate nucleus, the lateral septum, the anterior hypothalamus and the bed nucleus of the stria terminalis [29, 31, 32, 37]. Notably, RFRP-ir fibres make apparent contact with a subpopulation of GnRH neurons in rodents and sheep [26, 28, 29, 38, 39] suggesting that RFRP-3 acts centrally to control the reproductive axis (Table 2).

There is still a debate on a potential hypophysiotrophic effect of RFRP-3 in mammals as reported in birds. A large body of evidence now reports the absence of fibres in the median eminence of mice, rats and Siberian hamsters [26, 27, 31, 40]. In another study, only sparse RFRP fibre innervation was observed in the median eminence of mice, rats and Syrian hamsters [29] (Table 2). On the other hand, in the sheep RFRP fibres terminating in the median eminence have been identified and RFRP has been detected in the portal blood [35, 41, 42] (Table 2). These data suggest likely species-dependent differences in the modes of action of RFRP-3, and it is possible that sheep and rodents evolved this system differently.

The RFRP peptides bind with high affinity to GPR147 (also known as NPFF1R) and with a lower affinity to GPR74 (also known as NPFF2R), which were first identified as neuropeptide FF receptors [8, 18, 43]. The GPR147 receptor couples with $G\alpha_{i3}$ or $G_{\alpha s}$ proteins [44] suggesting that GPR147 can have both inhibitory and stimulatory downstream effects on cellular activity. However, in CHO cells, activation of the receptor inhibits forskolin-stimulated cAMP accumulation [45].

Table 2. Summary of the effects of RFRP-3 on LH secretion and of the sites of action of the peptide in mammals.

		Effects of RFRP-3 on the reproductive axis				Modes/sites of action			
Species	Sexual status	Effect of icv RFRP-3 on LH secretion	References	Effect of peripheral RFRP-3 on LH secretion	References	GnRH neurons	References	Pituitary	References
mice	male & female			no effect	[92]	RFRP-3 affects the firing rate of GnRH neurons RFRP fibres contact GnRH neurons	[49] [29] [38] [39]	sparse RFRP fibres in the median eminence	[29]
rats	male	inhibition	[32] [62]			RFRP fibres contact GnRH neurons	[29] [39]		
	female					RFRP-3 inhibits GnRH neuronal activation at the LH surge peak	[63]	sparse RFRP fibres in the median eminence RFRP-3 inhibits GnRH-induced LH secretion from cultured pituitary cells but not basal LH secretion in one study but had no effect in another	[29] [63] [64]
	male GNX	inhibition	[62]	inhibition	[62]			RFRP-3 inhibits LH secretion from cultured pituitary cells	[62]
	female OVX	inhibition in one study no effect in another	[62] [64]	inhibition	[64]				
Siberian hamster	male	long day conditions: inhibition short day conditions: stimulation	[26]			RFRP fibres contact GnRH neurons	[26]		

Table 2 continued..

Syrian hamster	male	long day conditions: stimulation short day conditions: reactivation of the reproductive axis	[25]	no effect	[25]	c-Fos expression in GnRH neurons following acute RFRP-3 administration	[25]	no effect	[25]
	female OVX	inhibition	[29]	inhibition	[29]	RFRP fibres contact GnRH neurons	[29]	sparse RFRP fibres in the median eminence	[29]
sheep	female OVX	inhibition in one study no effect in another	[35] [66]	inhibition in two studies no effect in another	[41] [35] [66]	RFRP fibres contact GnRH neurons	[28]	RFRP fibres terminating in the median eminence RFRP detected in portal blood	[42]
cattle	male GNX			inhibition	[65]			RFRP-3 inhibits LH secretion from cultured pituitary cells	[65]

NPFF receptors have been detected in rodent, lagomorph, and monkey brains suggesting that they are conserved [46]. Importantly, however, remarkable variations in GPR147 and GPR74 receptor contents and distribution exist from one species to another and from one strain to another among the same species [46, 47]. Early studies describing the autoradiographic distribution of GPR147 in mice and rats indicated that the receptor was present throughout the hypothalamus [46-48]. Recent studies have made it possible to localise RFRP sites of action in more detail in various rodent species. Indeed, RFRP-3 fibres are in contact with 20-40% of GnRH neurons in rats and hamsters [26, 39] and about 25% of GnRH neurons express *Gpr147*, but not *Gpr74* in mice, rats and hamsters [26, 38, 39]. In another study in mice expressing GnRH-green fluorescent protein-tagged neurons, RFRP-3 was found to exert a direct inhibitory effect on the firing rate of 41% of GnRH neurons, while 12% increased their firing rate, and the remaining were unaffected [49]. Furthermore, we demonstrated that central injection of RFRP-3 to Syrian hamsters induces c-Fos expression in 30% of the GnRH neurons [25]. Whether this effect is due to a direct action of RFRP-3 on GnRH neurons or whether it is linked to an effect on upstream regulators of the reproductive axis remains to be determined. Indeed, in the same study, although c-Fos expression was not observed in Kp neurons following acute RFRP-3 administration, the continuous central administration of RFRP-3 led to an increase in *Kiss1* expression in the arcuate nucleus [25]. Moreover, in rats RFRP-3 fibres are in contact with Kp neurons, of which a subpopulation (20%) expresses the *Gpr147* gene [39]. It is noteworthy that in our analysis of c-Fos expression in the Syrian hamster brain following icv RFRP-3 administration we found an increase in non-Kp neurons in the arcuate nucleus [25].

Only a few studies have addressed the question of the distribution of *Gpr147* in peripheral tissues. The receptor has been localised in the Syrian hamster pituitary [50] although only a very low level of pituitary expression has been reported in rats [8, 51]. More recently, ovine pituitary cells have been shown to express *Gpr147* [42]. These data further support the hypothesis that RFRP-3 could have a direct hypophysiotrophic effect in sheep and not in rodents, although additional studies will be required in order to provide an answer to this controversial question.

3. RFRP-3 and the reproductive axis

3.1. Lessons from non-mammalian vertebrates

As previously mentioned, GnIH was discovered in birds in 2000 and termed accordingly because of its inhibitory effect on gonadotrophin secretion [24]. Indeed, GnIH administration reduces plasma LH concentrations *in vivo* in quails and sparrows [24, 52, 53] and inhibits gonadotrophin synthesis and release *in vitro* from cultured quail and chicken pituitaries [53, 54]. Taken together, these data indicate that GnIH inhibits gonadotrophin synthesis and release in birds, likely via a direct inhibitory effect at the level of the pituitary; see [55-57] for review.

In 2002, goldfish GnIH was discovered in teleosts [58] leading to investigation of the involvement of this peptide in gonadotrophin secretion in fish. In goldfish, intraperitoneal administration of GnIH peptide induced a decrease in serum LH levels [59], however, goldfish GnIH and its related peptides stimulated the release of LH and FSH from cultured pituitary cells of sockeye salmon [60]. These results raise interesting questions on possible species-dependent differences in the effects of GnIH on gonadotrophin secretion in vertebrates. Additional studies should aim at determining whether these conflicting data are due to the different methods of investigation used, or whether they reflect a physiological reality in the effect of GnIH on the reproductive axis.

3.2. Actions of RFRP-3 on gonadotrophin synthesis and release in mammals

The discovery that GnIH was a potent regulator of gonadotrophin synthesis and release in non-mammalian vertebrates led to intensive research on the possible roles of RFRP-1 and RFRP-3 in the regulation of the mammalian reproductive axis. As RFRP-3 is closest to avian GnIH with regard to its sequence, focus was initially directed towards the role of RFRP-3 in the regulation of mammalian reproduction, to the detriment of RFRP-1. Moreover, an intial study in rats indicating that icv RFRP-1 stimulated prolactin secretion, but not other pituitary hormones [8], suggested that this peptide might be involved in the regulation of other endocrine functions rather than reproduction.

In recent years, a large number of studies have demonstrated in a range of mammalian species that RFRP-3 plays a role in the regulation of the hypothalamo-pituitary-gonadal axis (Table 2) (see [56, 61] for reviews).

In mice RFRP-3 was found to exhibit rapid and repeatable inhibitory effects on the firing rate of a subpopulation of GnRH neurons in hypothalamic slices [49]. In male rats, icv RFRP-3 significantly suppresses all facets of sex behaviour and also significantly reduces plasma levels of LH [32, 62]. In female rats, chronic icv infusion of RFRP-3 causes a dose-dependent inhibition of GnRH neuronal activation at the LH surge peak and also suppresses neuronal activation in the anteroventral periventricular region, which provides stimulatory input to GnRH neurons [63]. Taken together, these results point to a central inhibitory effect of RFRP-3 on the HPG axis, via the GnRH neurons in the POA/ OVLT brain region.

However, there are contradictory data about a possible hypophysiotrophic effect of the peptide in mammals. In ovariectomised (OVX) rats, intravenous administration of RFRP-3 significantly reduces plasma LH concentrations [64], while in another study the same protocol had no effect on basal LH secretion and minimal effects on GnRH-stimulated secretion [27]. *In vitro*, RFRP-3 was shown to inhibit LH secretion from cultured pituitary cells when GnRH is present, but did not have a significant effect on basal LH levels in the same study [64]. In another study, RFRP-3 did not have a direct suppressive effect on LH secretion in cultured rat anterior pituitary cells [63]. In OVX female Syrian hamsters, a study has shown that peripheral injections of GnIH significantly inhibit LH secretion, but in the male hamster we reported no effect of RFRP-3 on LH secretion when injected peripherally, or on the basal or GnRH-stimulated production of LH from isolated pituitary glands [25]. In sheep and cattle, intravenous RFRP-3 administration inhibits gonadotrophin release [35, 65], although another study failed to replicate these results in the sheep [66]. Interestingly, RFRP-3 is released into the portal blood in sheep and appears to induce a marked inhibition of gonadotrophin secretion *in vitro* [35, 41, 42]. To date, no consensus has been reached on the subject of RFRP-3 sites of action for the control of mammalian reproduction and it is possible that species-dependent differences exist with regard to the hypophysiotrophic effect of RFRP-3 in mammals. Additional studies, using similar experimental protocols in rodents and sheep could help to answer some of the pending questions.

Until recently, and based on the plethora of publications supporting this hypothesis, it was assumed that RFRP-3 functioned in mammals as GnIH functioned in birds and served as an inhibitory component regulating the hypothalamo-pituitary-gonadal axis. However, we have recently reported novel findings in the male Syrian hamster [25] which have led to call this assumption into question, concurrently with another group working on the male Siberian hamster [26]. In the male Syrian hamster kept in long-day photoperiodic (LD) conditions, we reported that acute icv administration of RFRP-3 stimulates GnRH cell activity, gonadotrophin release and testosterone production [25]. In the same manner, in short-day photoperiodic (SD) conditions, a single central injection of RFRP-3 increases gonadotrophin release (unpublished data). In the Siberian hamster, while administration of RFRP-3 in LD conditions inhibits gonadotrophin release, the same protocol stimulates gonadotrophin secretion in SD conditions [26]. Remarkably, these findings of a stimulatory action of RFRP-3 on the male hamster reproductive axis are in sharp contrast with a previous study reporting an inhibitory effect of icv GnIH on LH secretion in OVX female Syrian hamsters [29], raising the question of a possible sex-dependent difference in the effect of RFRP-3 on the reproductive axis.

Reproductive activity of female rodents displays a well-described oestrous cycle, characterised by varying levels of circulating gonadotrophins and sex steroids. It has been hypothesised that the RFRP neuronal system might be involved in the estrogen-mediated positive feedback which regulates the oestrous cycle. Indeed, the number of RFRP neurons and their level of activity are decreased at the time of the LH surge in the Syrian hamster [50]. Furthermore, *rfrp* mRNA expression is reduced in OVX mice treated with estrogen [67]. However, a study in rats showed no difference in *rfrp* mRNA levels of females that were OVX, versus OVX and treated with estrogen or diestrus [51]. In addition, in OVX ewes, estrogen treatment does not significantly

alter *rfrp* mRNA expression levels [28]. These observations suggest that there could be another level of complexity in the involvement of the RFRP neuronal system in the regulation of the reproductive system, according to the gender of the animal analyzed. In this context it would be interesting to determine whether the effect of RFRP-3 on the female reproductive axis depends on the stage of the oestrous cycle at which it is administered.

3.3. RFRP-3 and seasonal reproduction

In seasonal breeders, reproduction is restricted to a specific time of the year to ensure that the birth of the offspring occurs during the most favourable season. In order to synchronise their reproductive activity with the seasons, mammals use the annual variations in photoperiod. To decode photoperiod, mammals rely on a photoneuroendocrine system in which cells originating in the retina project, via a multisynaptic pathway, to the pineal gland, where melatonin is released exclusively at night. As a result, the duration of the nocturnal release of melatonin is proportional to the night duration, thus giving a stable indication of the seasons; see [68] for review.

Syrian and Siberian hamsters are classic models for the study of seasonal rhythms. In these species, sexual activity is promoted by exposure to a LD and exposure to a SD induces an inhibition of the reproductive function within 8-10 weeks. Although it is now well established that the seasonal regulation of reproduction is mediated via melatonin, its precise sites of action remain unknown.

In the Syrian hamster, the mediobasal hypothalamus appears as an important brain region in the photoperiodic control of reproduction. Indeed, melatonin receptors are localised in this hypothalamic area and an electrolytic lesion of the mediobasal hypothalamus prevents the SD-induced gonadal regression [69, 70]. Interestingly, *rfrp* neurons are localised in this same brain region and we have shown that both *rfrp* mRNA and RFRP protein levels are down-regulated by melatonin in a SD photoperiod in the Syrian hamster [34]. Recently, a similar melatonin-driven down-regulation of *rfrp* mRNA levels and RFRP-ir content has been reported in the male Siberian hamster [26]. It is worth noting that in both hamster species these photoperiodic variations of *rfrp* expression are independent of the photoperiodic variation in circulating levels of testosterone. Taken together, these data suggest that *rfrp* and its product, RFRP-3, might be involved in the melatonin-driven seasonal regulation of reproduction in hamsters.

We have recently investigated the role of RFRP-3 in the seasonal control of reproduction. Male Syrian hamsters were placed in photoinhibitory conditions and implanted with osmotic minipumps releasing a constant flow of RFRP-3 in the lateral ventricle. Within 5 weeks, RFRP-3 administration had fully reactivated the reproductive function compared to the administration of vehicle, manifested by an increase in *Kiss1* expression in the arcuate nucleus, paired testes weight and plasma testosterone concentrations [25]. These results indicate that *rfrp* neurons are likely candidates in mediating the melatonergic information to the reproductive axis. However, additional experiments are required in order to determine whether melatonin is acting directly upon *rfrp* neurons or whether there are other intermediates involved in the melatonin-driven regulation of the reproductive function. In particular, the pars tuberalis has been proposed to play a central role in the photoperiodic control of seasonal functions. Indeed, in seasonal species melatonin receptors are present abundantly in the pars tuberalis of the anterior pituitary, and melatonin-responsive cells in the pars tuberalis control the production of thyrotrophin which acts locally on cells in the adjacent mediobasal hypothalamus, leading to increased expression of type II thyroid hormone deiodinase (Dio2) in LD conditions in Syrian and Siberian hamsters [71, 72]. As Dio2 catalyzes the conversion of thyroxine (T_4) to the bioactive form triiodothyronine (T_3), this photoperiodic regulation results in elevated levels of T_3 during the breeding season, compared to the non-breeding season. Interestingly, in photoinhibited Siberian hamsters T_3 administration reactivates the reproductive function [73], indicating that this pathway could be involved in the regulation of seasonal reproduction. Additional studies investigating the effect of T_3 administration on RFRP expression and the presence or not of T_3 receptors on RFRP neurons could help clarify the hierarchical organization of the T_3/RFRP systems.

Contrary to hamsters, sheep are short day breeders; this is to say that sexual activity is promoted by

exposure to a SD photoperiod and inhibited upon exposure to a LD photoperiod. In this species, as hamsters, *rfrp* expression is down-regulated in SD conditions, when sheep are sexually active, and elevated in LD conditions, when they are sexually inactive [28, 36]. These observations are in line with the findings indicating that acute administration of RFRP-3 has an inhibitory effect on the reproductive function in sheep [35, 41, 65]. However, a possible seasonal role has to date not been addressed using continuous infusions of the peptide. In the future, it will be interesting to determine whether RFRP-3 is a regulator of seasonal reproduction in sheep as it appears to be the case in hamsters. Moreover, as previously mentioned *rfrp* neurons are likely candidates in mediating the melatonergic information to the reproductive axis in hamsters, and it will be fascinating to find out whether they play a central role in transmitting seasonal information to the gonadotrophic axis in sheep. Indeed, although it is well established that melatonin controls the seasonal regulation of the hypothalamo-pituitary-gonadal axis in seasonally-breeding species, the precise mechanisms through which the same melatoninergic signal produces opposite behavioural responses remain unclear. It is reasonable to hypothesise that RFRP neurons are the switch points in converting the same melatoninergic signal into a stimulatory or an inhibitory output to the reproductive axis in seasonally-breeding mammals.

4. RFRPs and other functions

The DMH is involved in a variety of behavioral and physiological responses. Thus the involvement of RFRPs in the regulation of other endocrine functions has been investigated. Interestingly, the peptides have been found to be implicated in functions which indirectly affect or are affected by the reproductive status of the animal, including feeding, stress and nociception.

4.1. RFRPs and feeding

As the DMH plays an important role in the control of energy metabolism and RFRP neurons are located in the DMH in mammalian species, it seems likely that RFRP-1 and/or RFRP-3 may play a role in the regulation of feeding behaviour. In the sheep brain, RFRP fibres are found to have close appositions with neuropeptide Y, proopiomelanocortin, orexin, and melanin-concentrating hormone neurons [30], all of which are known to play important roles in the control of food intake. Moreover, the administration of RFRPs induces c-Fos expression in the arcuate nucleus in rats and hamsters [25, 40], a brain region well-known for its key role in the regulation of feeding behaviour.

Only a few studies have investigated the behavioural effect of RFRP peptide injections in mammals. In rats, icv RFRP-3 administration induces an increase in food intake [32, 64] and in body weight [74]. However, in another study central RFRP-1 injection resulted in food intake decrease in rats [75]. Given that RFRP-1 applied icv to chicks significantly reduced both food intake and water intake [76], it is reasonable to speculate that RFRP-1 and RFRP-3 might have variable effects on food intake in mammals. Indeed, in the Syrian hamster we have shown that continuous icv administration of RFRP-3 for 5 weeks had no impact on food intake or body weight [25].

4.2. RFRPs and stress

The DMH is also involved in the control of stress responses [77] and the involvement of RFRP in the control of stress has been reported. Exposure to stressful stimuli induces an increase in *rfrp* expression and an activation of RFRP neurons in the hypothalamus [78, 79]. Moreover, RFRP fibers appear to project directly to cells containing corticotrophin-releasing hormone or oxytocin in the hypothalamus, hormones which are known for their role in stress responses [30]. Administration of RFRP-1 and RFRP-3 induces c-Fos expression in the hypothalamic paraventricular nucleus and in oxytocin neurons, and induces the secretion of adrenocorticotropic hormone and oxytocin into the peripheral circulation [78]. Interestingly, similar patterns of c-Fos expression and hormone release are observed after stressful stimuli [80]. In addition, central administration of RFRP induces anxiety-related behaviors [78]. On the other hand, initial work indicated that central RFRP-1 application increased prolactin secretion in rats [8]. Given the anti-stress and anxiolytic properties of prolactin, this aspect of RFRP peptide function deserves further investigation. Taken together, these data are consistent with the view that RFRPs are involved

in neuroendocrine and behavioral responses to stressful stimuli.

4.3. RFRPs and nociception

Two peptides of the RFamide family of peptides have been shown to play important roles in the control of pain and analgesia, namely NPFF and NPAF; see [17, 81, 82] for review. Interestingly, these peptides were initially identified as the endogenous ligands for GPR74 and GPR147 [8, 83, 84], but it was later shown that NPFF and NPAF had a lower affinity for GPR147 than RFRP peptides [8, 18, 43]. In 2001, a study showing that RFRP-1 is more potent in attenuating morphine-induced analgesia than NPFF when injected icv [18] suggested that RFRP peptides could play a role in nociception. More recently, using neuroblastoma cells transfected with GPR147 a similar opioid-attenuating activity was observed for RFRP-3 [85], further supporting the possibility that RFRP peptides are involved in the control of pain and analgesia. Unfortunately, only a few studies have aimed at clarifying this aspect of RFRP peptide function, and it is therefore difficult to conclude on the implication of these peptides in nociception. However, a recent report indicating that RF9, a dipeptide with subnanomolar affinities towards GPR147, exhibited a potent *in vivo* preventive effect on opioid-induced hyperalgesia at low dose [86] indicates that GPR147 may be a key partner of an anti-opioid system that modulates the antinociceptive properties of the opioid system. Since the endogenous ligands for GPR147 are RFRP peptides, this branch of research deserves further investigation and might lead to the discovery of an additional function for RFRP peptides, besides the regulation of the gonadotrophic axis.

5. CONCLUSION AND PERSPECTIVES

The discovery of *rfrp* and its product RFRP-3 in mammals led to a new direction in investigating the regulation of GnRH neuron activity and therefore of the HPG axis. Although it was initially hypothesised that RFRP-3 would act as an inhibitory component regulating the reproductive axis in mammals, it now appears that this is not always the case. Indeed, recent evidence indicates that there are likely species-dependent differences in the effect of the peptide on the gonadotrophic axis. Further investigations will be required in order to answer the questions raised by the contradictory results observed in mammals, notably: 1) what is the functional significance of these opposing effects? 2) through which mechanisms does RFRP-3 induce either a stimulatory or an inhibitory effect on the gonadotrophic axis? 3) apart from GnRH neurons, what are the central sites of action of the peptide? and 4) to what extent is RFRP-3 involved in the regulation of non-reproductive functions?

The future development of highly selective pharmacological and molecular tools should help answer the question as to whether the species- and gender-dependent differences in the physiological effects of RFRP-3 might be mediated by differences in the modes and sites of action of the peptide on the HPG axis.

ACKNOWLEDGEMENTS

We thank Dr. Jens D. Mikkelsen for valuable comments on an earlier vesion of this manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

REFERENCES

1. de Roux, N., Genin, E., Carel, J. C., Matsuda, F., Chaussain, J. L. and Milgrom, E. 2003, Proc. Natl. Acad. Sci. USA, 100, 10972-6.
2. Seminara, S. B., Messager, S., Chatzidaki, E. E., Thresher, R. R., Acierno, J. S. Jr., Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwinof, K. M., Hendrick, A. G., Zahn, D., Dixon, J., Kaiser, U. B., Slaugenhaupt, S. A., Gusella, J. F., O'Rahilly, S., Carlton, M. B., Crowley, W. F. Jr., Aparicio, S. A. and Colledge, W. H. 2003, N. Engl. J. Med., 349, 1614-27.
3. Irwig, M. S., Fraley, G. S., Smith, J. T., Acohido, B. V., Popa, S. M., Cunningham, M. J., Gottsch, M. L., Clifton, D. K. and Steiner, R. A. 2004, Neuroendocrinology, 80, 264-72.
4. Han, S. K., Gottsch, M. L., Lee, K. J., Popa, S. M., Smith, J. T., Jakawich, S. K., Clifton, D. K., Steiner, R. A. and Herbison, A. E. 2005, J. Neurosci., 25, 11349-56.

5. Ramaswamy, S., Guerriero, K. A., Gibbs, R. B. and Plant, T. M. 2008, Endocrinology, 149, 4387-95.
6. d'Anglemont de Tassigny, X., Fagg, L. A., Carlton, M. B. and Colledge, W. H. 2008, Endocrinology, 149, 3926-32.
7. Pinilla, L., Aguilar, E., Dieguez, C., Millar, R. P. and Tena-Sempere, M. 2012, Physiol. Rev., 92, 1235-316.
8. Hinuma, S., Shintani, Y., Fukusumi, S., Iijima, N., Matsumoto, Y., Hosoya, M., Fujii, R., Watanabe, T., Kikuchi, K., Terao, Y., Yano, T., Yamamoto, T., Kawamata, Y., Habata, Y., Asada, M., Kitada, C., Kurokawa, T., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y. and Fujino, M. 2000, Nat. Cell Biol., 2, 703-8.
9. Price, D. A. and Greenberg, M. J. 1977, Prep. Biochem., 7, 261-81.
10. Walker, R. J. 1992, Comp. Biochem. Physiol. C, 102, 213-22.
11. Dockray, G. J., Reeve, J. R. Jr., Shively, J., Gayton, R. J. and Barnard, C. S. 1983, Nature, 305, 328-30.
12. Price, D. A. and Greenberg, M. J. 1977, Science, 197, 670-1.
13. Barnard, C. S. and Dockray, G. J. 1984, Regul. Pept., 8, 209-15.
14. Yang, H. Y., Fratta, W., Majane, E. A. and Costa, E. 1985, Proc. Natl. Acad. Sci. USA, 82, 7757-61.
15. Perry, S. J., Yi-Kung Huang, E., Cronk, D., Bagust, J., Sharma, R., Walker, R. J., Wilson, S. and Burke, J. F. 1997, FEBS Lett, 409, 426-30.
16. Hinuma, S., Habata, Y., Fujii, R., Kawamata, Y., Hosoya, M., Fukusumi, S., Kitada, C., Masuo, Y., Asano, T., Matsumoto, H., Sekiguchi, M., Kurokawa, T., Nishimura, O., Onda, H. and Fujino, M. 1998, Nature, 393, 272-6.
17. Panula, P., Kalso, E., Nieminen, M., Kontinen, V. K., Brandt, A. and Pertovaara, A. 1999, Brain Res., 848, 191-6.
18. Liu, Q., Guan, X. M., Martin, W. J., McDonald, T. P., Clements, M. K., Jiang, Q., Zeng, Z., Jacobson, M., Williams, D. L. Jr., Yu, H., Bomford, D., Figueroa, D., Mallee, J., Wang, R., Evans, J., Gould, R. and Austin, C. P. 2001, J. Biol. Chem., 276, 36961-9.
19. Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J. M., Le Poul, E., Brezillon, S., Tyldesley, R., Suarez-Huerta, N., Vandeput, F., Blanpain, C., Schiffmann, S. N., Vassart, G. and Parmentier, M. 2001, J. Biol. Chem., 276, 34631-6.
20. Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., Terao, Y., Kumano, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Onda, H., Nishimura, O. and Fujino, M. 2001, Nature, 411, 613-7.
21. Chartrel, N., Dujardin, C., Anouar, Y., Leprince, J., Decker, A., Clerens, S., Do-Rego, J. C., Vandesande, F., Llorens-Cortes, C., Costentin, J., Beauvillain, J. C. and Vaudry, H. 2003, Proc. Natl. Acad. Sci. USA, 100, 15247-52.
22. Fukusumi, S., Yoshida, H., Fujii, R., Maruyama, M., Komatsu, H., Habata, Y., Shintani, Y., Hinuma, S. and Fujino, M. 2003, J. Biol. Chem., 278, 46387-95.
23. Jiang, Y., Luo, L., Gustafson, E. L., Yadav, D., Laverty, M., Murgolo, N., Vassileva, G., Zeng, M., Laz, T. M., Behan, J., Qiu, P., Wang, L., Wang, S., Bayne, M., Greene, J., Monsma, F. Jr. and Zhang, F. L. 2003, J. Biol. Chem., 278, 27652-7.
24. Tsutsui, K., Saigoh, E., Ukena, K., Teranishi, H., Fujisawa, Y., Kikuchi, M., Ishii, S. and Sharp, P. J. 2000, Biochem. Biophys. Res. Commun., 275, 661-7.
25. Ancel, C., Bentsen, A. H., Sebert, M. E., Tena-Sempere, M., Mikkelsen, J. D. and Simonneaux, V. 2012, Endocrinology, 153, 1352-63.
26. Ubuka, T., Inoue, K., Fukuda, Y., Mizuno, T., Ukena, K., Kriegsfeld, L. J. and Tsutsui, K. 2012, Endocrinology, 153(1), 373-85.
27. Rizwan, M. Z., Porteous, R., Herbison, A. E. and Anderson, G. M. 2009, Endocrinology, 150, 1413-20.
28. Smith, J. T., Coolen, L. M., Kriegsfeld, L. J., Sari, I. P., Jaafarzadehshirazi, M. R., Maltby, M., Bateman, K., Goodman, R. L., Tilbrook, A. J., Ubuka, T., Bentley, G. E., Clarke, I. J. and Lehman, M. N. 2008, Endocrinology, 149, 5770-82.

29. Kriegsfeld, L. J., Mei, D. F., Bentley, G. E., Ubuka, T., Mason, A. O., Inoue, K., Ukena, K., Tsutsui, K. and Silver, R. 2006, Proc. Natl. Acad. Sci. USA, 103, 2410-5.
30. Qi, Y., Oldfield, B. J. and Clarke, I. J. 2009, J. Neuroendocrinol., 21, 690-7.
31. Ukena, K. and Tsutsui, K. 2001, Neurosci. Lett., 300, 153-6.
32. Johnson, M. A., Tsutsui, K. and Fraley, G. S. 2007, Hormones and Behavior, 51, 171-80.
33. Legagneux, K., Bernard-Franchi, G., Poncet, F., La Roche, A., Colard, C., Fellmann, D., Pralong, F. and Risold, P. Y. 2009, Neuropeptides, 43, 13-9.
34. Revel, F. G., Saboureau, M., Pevet, P., Simonneaux, V. and Mikkelsen, J. D. 2008, Endocrinology, 149, 902-12.
35. Clarke, I. J., Sari, I. P., Qi, Y., Smith, J. T., Parkington, H. C., Ubuka, T., Iqbal, J., Li, Q., Tilbrook, A., Morgan, K., Pawson, A. J., Tsutsui, K., Millar, R. P. and Bentley, G. E. 2008, Endocrinology, 149, 5811-21.
36. Dardente, H., Birnie, M., Lincoln, G. A. and Hazlerigg, D. G. 2008, J. Neuroendocrinol., 20, 1252-9.
37. Mason, A. O., Duffy, S., Zhao, S., Ubuka, T., Bentley, G. E., Tsutsui, K., Silver, R. and Kriegsfeld, L. J. 2010, J. Biol. Rhythms., 25, 176-85.
38. Poling, M. C., Kim, J., Dhamija, S. and Kauffman, A. S. 2012, Endocrinology, 153, 1827-40.
39. Rizwan, M. Z., Poling, M. C., Corr, M., Cornes, P. A., Augustine, R. A., Quennell, J. H., Kauffman, A. S. and Anderson, G. M. 2012, Endocrinology, 153, 3770-9.
40. Yano, T., Iijima, N., Kakihara, K., Hinuma, S., Tanaka, M. and Ibata, Y. 2003, Brain Res., 982, 156-67.
41. Sari, I. P., Rao, A., Smith, J. T., Tilbrook, A. J. and Clarke, I. J. 2009, Endocrinology, 150, 5549-56.
42. Smith, J. T., Young, I. R., Veldhuis, J. D. and Clarke, I. J. 2012, Endocrinology, 153, 3368-75.
43. Engstrom, M., Brandt, A., Wurster, S., Savola, J. M. and Panula, P. 2003, J. Pharmacol. Exp. Ther., 305, 825-32.
44. Gouarderes, C., Mazarguil, H., Mollereau, C., Chartrel, N., Leprince, J., Vaudry, H. and Zajac, J. M. 2007, Neuropharmacology, 52, 376-86.
45. Mollereau, C., Mazarguil, H., Marcus, D., Quelven, I., Kotani, M., Lannoy, V., Dumont, Y., Quirion, R., Detheux, M., Parmentier, M. and Zajac, J. M. 2002, Eur. J. Pharmacol., 451, 245-56.
46. Gouarderes, C., Puget, A. and Zajac, J. M. 2004, Synapse, 51, 249-69.
47. Gouarderes, C., Faura, C. C. and Zajac, J. M. 2004, Brain Res., 1014, 61-70.
48. Gouarderes, C., Quelven, I., Mollereau, C., Mazarguil, H., Rice, S. Q. and Zajac, J. M. 2002, Neuroscience, 115, 349-61.
49. Ducret, E., Anderson, G. M. and Herbison, A. E. 2009, Endocrinology, 150, 2799-804.
50. Gibson, E. M., Humber, S. A., Jain, S., Williams, W. P. 3rd, Zhao, S., Bentley, G. E., Tsutsui, K. and Kriegsfeld, L. J. 2008, Endocrinology, 149, 4958-69.
51. Quennell, J. H., Rizwan, M. Z., Relf, H. L. and Anderson, G. M. 2010, J. Neuroendocrinol., 22, 309-16.
52. Osugi, T., Ukena, K., Bentley, G. E., O'Brien, S., Moore, I. T., Wingfield, J. C. and Tsutsui, K. 2004, J. Endocrinol., 182, 33-42.
53. Ubuka, T., Ukena, K., Sharp, P. J., Bentley, G. E. and Tsutsui, K. 2006, Endocrinology, 147, 1187-94.
54. Ciccone, N. A., Dunn, I. C., Boswell, T., Tsutsui, K., Ubuka, T., Ukena, K. and Sharp, P. J. 2004, J. Neuroendocrinol., 16, 999-1006.
55. Tsutsui, K., Saigoh, E., Yin, H., Ubuka, T., Chowdhury, V. S., Osugi, T., Ukena, K., Sharp, P. J., Wingfield, J. C. and Bentley, G. E. 2009, J. Neuroendocrinol., 21, 271-5.
56. Tsutsui, K., Bentley, G. E., Bedecarrats, G., Osugi, T., Ubuka, T. and Kriegsfeld, L. J. 2010, Front Neuroendocrinol., 31, 284-95.
57. Tsutsui, K., Ubuka, T., Bentley, G. E. and Kriegsfeld, L. J. 2012, Gen. Comp. Endocrinol., 177, 305-14.
58. Sawada, K., Ukena, K., Satake, H., Iwakoshi, E., Minakata, H. and Tsutsui, K. 2002, Eur. J. Biochem., 269, 6000-8.
59. Zhang, Y., Li, S., Liu, Y., Lu, D., Chen, H., Huang, X., Liu, X., Meng, Z., Lin, H. and Cheng, C. H. 2010, Peptides, 31, 1034-43.
60. Amano, M., Moriyama, S., Iigo, M., Kitamura, S., Amiya, N., Yamamori, K. and Ukena, K. and Tsutsui, K. 2006, J. Endocrinol., 188, 417-23.
61. Bentley, G. E., Tsutsui, K. and Kriegsfeld, L. J. 2010, Brain Research, 1364, 62-71.

62. Pineda, R., Garcia-Galiano, D., Sanchez-Garrido, M. A., Romero, M., Ruiz-Pino, F., Aguilar, E., Dijcks, F. A., Blomenrohr, M., Pinilla, L., van Noort, P. I. and Tena-Sempere, M. 2010, Am. J. Physiol. Endocrinol. Metab., 299, E39-46.
63. Anderson, G. M., Relf, H. L., Rizwan, M. Z. and Evans, J. J. 2009, Endocrinology, 150, 1834-40.
64. Murakami, M., Matsuzaki, T., Iwasa, T., Yasui, T., Irahara, M., Osugi, T. and Tsutsui, K. 2008, J. Endocrinol., 199, 105-12.
65. Kadokawa, H., Shibata, M., Tanaka, Y., Kojima, T., Matsumoto, K., Oshima, K. and Yamamoto, N. 2009, Domestic Animal Endocrinology, 36, 219-24.
66. Caraty, A., Blomenrohr, M., Vogel, G. M., Lomet, D., Briant, C. and Beltramo, M. 2012, J. Neuroendocrinol., 24, 725-36.
67. Molnar, C. S., Kallo, I., Liposits, Z. and Hrabovszky, E. 2011, Endocrinology, 152(4), 1684-1690.
68. Simonneaux, V. and Ribelayga, C. 2003, Pharmacol. Rev., 55, 325-95.
69. Maywood, E. S. and Hastings, M. H. 1995, Endocrinology, 136, 144-53.
70. Maywood, E. S., Bittman, E. L. and Hastings, M. H. 1996, Biol. Reprod., 54, 470-7.
71. Revel, F. G., Saboureau, M., Pevet, P., Mikkelsen, J. D. and Simonneaux, V. 2006, Endocrinology, 147, 4680-7.
72. Watanabe, M., Yasuo, S., Watanabe, T., Yamamura, T., Nakao, N., Ebihara, S. and Yoshimura, T. 2004, Endocrinology, 145, 1546-9.
73. Freeman, D. A., Teubner, B. J., Smith, C. D. and Prendergast, B. J. 2007, Am. J. Physiol. Regul. Integr. Comp. Physiol., 292, R2368-72.
74. Johnson, M. A. and Fraley, G. S. 2008, Neuroendocrinology, 88, 305-15.
75. Kovacs, A., Laszlo, K., Galosi, R., Toth, K., Ollmann, T., Peczely, L. and Lenard, L. 2012, Brain Res. Bull., 88, 589-95.
76. Newmyer, B. A. and Cline, M. A. 2009, Behav. Brain Res., 205, 311-4.
77. DiMicco, J. A., Samuels, B. C., Zaretskaia, M. V. and Zaretsky, D. V. 2002, Pharmacol. Biochem. Behav., 71, 469-80.
78. Kaewwongse, M., Takayanagi, Y. and Onaka, T. 2010, J. Neuroendocrinol., 23, 20-7.
79. Kirby, E. D., Geraghty, A. C., Ubuka, T., Bentley, G. E. and Kaufer, D. 2009, Proc. Natl. Acad. Sci. USA, 106, 11324-9.
80. Onaka, T. 2000, Exp. Physiol., 85 Spec No, 101S-10S.
81. Panula, P., Aarnisalo, A. A. and Wasowicz, K. 1996, Prog. Neurobiol., 48, 461-87.
82. Roumy, M. and Zajac, J. M. 1998, Eur. J. Pharmacol., 345, 1-11.
83. Elshourbagy, N. A., Ames, R. S., Fitzgerald, L. R., Foley, J. J., Chambers, J. K., Szekeres, P. G., Evans, N. A., Schmidt, D. B., Buckley, P. T., Dytko, G. M., Murdock, P. R., Milligan, G., Groarke, D. A., Tan, K. B., Shabon, U., Nuthulaganti, P., Wang, D. Y., Wilson, S., Bergsma, D. J. and Sarau, H. M. 2000, J. Biol. Chem., 275, 25965-71.
84. Bonini, J. A., Jones, K. A., Adham, N., Forray, C., Artymyshyn, R., Durkin, M. M., Smith, K. E., Tamm, J. A., Boteju, L. W., Lakhlani, P. P., Raddatz, R., Yao, W. J., Ogozalek, K. L., Boyle, N., Kouranova, E. V., Quan, Y., Vaysse, P. J., Wetzel, J. M., Branchek, T. A., Gerald, C. and Borowsky, B. 2000, J. Biol. Chem., 275, 39324-31.
85. Kersante, F., Mollereau, C., Zajac, J. M. and Roumy, M. 2006, Peptides, 27, 980-9.
86. Gealageas, R., Schneider, S., Humbert, J. P., Bertin, I., Schmitt, M., Laboureyras, E., Dugave, C., Mollereau, C., Simonnet, G., Bourguignon, J. J., Simonin, F. and Bihel, F. 2012, Bioorg. Med. Chem. Lett., 22, 7471-4.
87. Ubuka, T., Morgan, K., Pawson, A. J., Osugi, T., Chowdhury, V. S., Minakata, H., Tsutsui, K., Millar, R. P. and Bentley, G. E. 2009, PLoS One, 4, e8400.
88. Fukusumi, S., Habata, Y., Yoshida, H., Iijima, N., Kawamata, Y., Hosoya, M., Fujii, R., Hinuma, S., Kitada, C., Shintani, Y., Suenaga, M., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y., Fujino, M. 2001, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1540, 221-32.
89. Yoshida, H., Habata, Y., Hosoya, M., Kawamata, Y., Kitada, C. and Hinuma, S. 2003, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1593, 151-7.
90. Ukena, K., Iwakoshi, E., Minakata, H. and Tsutsui, K. 2002, FEBS Lett, 512, 255-8.
91. Satake, H., Hisada, M., Kawada, T., Minakata, H., Ukena, K. and Tsutsui, K. 2001, Biochem. J., 354, 379-85.
92. Mikkelsen, J. D., Bentsen, A. H., Ansel, L., Simonneaux, V. and Juul, A. 2009, Regul. Pept., 152, 95-100.