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REGULAR ARTICLE

Expression of urocortin and corticotropin-releasing hormone receptors in the horse thyroid gland

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Abstract Urocortin (UCN) is a 40-amino-acid peptide and a member of the corticotropin-releasing hormone (CRH) family, which includes CRH, urotensin I, sauvagine, UCN2 and UCN3. The biological actions of CRH family peptides are mediated via two types of G-protein-coupled receptors, namely CRH type 1 receptor (CRHR1) and CRH type 2 receptor (CRHR2). The biological effects of these peptides are mediated and modulated not only by CRH receptors but also via a highly conserved CRH-binding protein (CRHBP). Our aim was to investigate the expression of UCN, CRHR1, CRHR2 and CRHBP by immunohistochemistry, Western blot and reverse transcription with the polymerase chain reaction (RT-PCR) in the horse thyroid gland. The results showed that UCN, CRHR1 and CRHR2 were expressed in the thyroid gland, whereas CRHBP was not expressed. Specifically, UCN immunoreactivity (-IR) was found in the thyroid follicular cells, CRHR2-IR in the C-cells and CRHR1-IR in blood vessels. Western blot analysis and RT-PCR experiments confirmed the immunohistochemical data. These results suggest that a regulatory system exists in the mammalian thyroid gland based on UCN, CRHR1 and CRHR2 and that UCN plays a role in the regulation of thyroid physiological functions through a paracrine mechanism.

Keywords Follicular cells · C-cells · RT-PCR · Immunohistochemistry · Horse

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Introduction

Urocortin (UCN) is a peptide of 40 amino acids and is a member of the corticotropin-releasing hormone (CRH) family, which includes CRH, urotensin I, sauvagine, UCN2 and UCN3. Vaughan et al. (1995) were the first to identify UCN, which exhibits 45% homology to CRH (Latchman 2002; Donaldson et al. 1996; Fekete and Zorrilla 2007). Like CRH, UCN can stimulate adrenocorticotrophic hormone (ACTH) production from anterior pituitary corticotrophs in vitro and in vivo (Asaba et al. 1998).

UCN is more potent than CRH with regard to other biological effects, including the suppression of appetite but is less potent in generating anxiety. A few years after the identification of UCN, two additional mammalian CRH-like peptides were isolated. UCN2 (stresscopin-related peptide) is a 38-amino-acid peptide with an amidated C-terminus and was first predicted in mouse from a gene encoding a 112-amino-acid protein (Reyes et al. 2001; Hsu and Hsueh 2001; Yamauchi et al. 2005). Human UCN3 (stresscopin) was identified later and found to have high identity (>80%) to UCN2 (Lewis et al. 2001; Takahashi et al. 2004; Saruta et al. 2005; Imperatore et al. 2006). The biological effects of CRH and UCNs are mediated by two distinct receptors, namely CRH receptor types 1 (CRHR1) and 2 (CRHR2), which belong to the G-protein-coupled receptor superfamily of brain-gut neuropeptides (Perrin et al. 1993; Vita et al.

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1995; Chen et al. 1993; Perrin et al. 2006). Two separate genes encode the CRH receptors (Chen et al. 1993). CRHR1, a 415-amino-acid protein, exhibits high affinity towards CRH and UCN but low affinity towards UCN2 and no affinity towards UCN3. CRHR1 is primarily expressed in the central nervous system (CNS) and the anterior pituitary. The CRHR2 receptor shares 70% sequence identity with CRHR1 and is expressed primarily in extra-CNS sites. CRHR2 receptors exhibit high affinity towards UCNs and no affinity towards CRH. Activation of CRHR2 suppresses multiple metabolic functions, including feeding in fasted mice (Bale et al. 2000), heat-induced oedema and gastric emptying (Kishimoto et al. 2000).

The biological effects of UCN and other CRH-related ligands are mediated and controlled not only by CRH receptors but also by the CRH-binding protein (CRHBP). The CRHBP is a 37-kDa glycoprotein identified not only in mammals but also in non-mammalian vertebrates including fishes, amphibians and birds, suggesting that it is a phylogenetically ancient protein with extensive structural and functional conservation (Seasholtz et al. 2002). It was originally isolated from human plasma (Orth and Mount 1987; Behan et al. 1989) and then found in several rat brain areas including the anterior pituitary gland (Potter et al. 1992; Peto et al. 1999). It is distinct from the CRH receptors and binds hCRH and UCN with an equal or greater affinity than the CRH receptors (Sutton et al. 1995; Vaughan et al. 1995). A major function of CRHBP is to modulate the access of CRH to CRHRs (Seasholtz et al. 2002).

The expression of UCN and CRHRs is not limited to the brain, as recent studies have detected immunoreactivity and mRNA for UCNs and their receptors in the digestive, cardiovascular, reproductive, immune and endocrine systems suggesting important roles for these factors throughout the body (Oki and Sasano 2004; Yang et al. 2010; Venkatasubramanian et al. 2010; Squillacioti et al. 2011).

Despite this evidence, no indications are available concerning the expression of CRH-related peptides in the normal thyroid gland. CRH, UCN 1 and UCN 3, however, have been found to be expressed in thyroid medullary carcinomas (Kageyama et al. 2008). CRH immunoreactivity (CRH-IR), in addition, has been detected in thyroid autoimmune and neoplastic lesions such as Hashimoto thyroiditis, neoplasms and goiters, suggesting that CRH may be directly and/or indirectly involved with inflammatory processes taking place in this gland (Scopa et al. 1994).

The aim of the present study has been to investigate the expression of UCN, CRHR1, CRHR2 and CRHBP in the horse thyroid gland to verify the existence of a regulatory system based on UCN, CRHRs and CRHBP in normal mammalian thyroid tissues. For this purpose, we have performed Western blot and immunohistochemistry to study the presence and distribution of these proteins and reverse transcription with the polymerase chain reaction (RT-PCR) to evaluate mRNA expression in the thyroid gland of the horse.

Materials and methods

Animals and tissue collection

This study was performed on six adult healthy horses of both sexes coming from the same farm located in southern Italy. The animals were bred under natural environmental conditions and were fed hay and concentrate. Water was available continuously. The animals were killed in a public slaughterhouse according to current Italian animal care protocols. Thyroid glands from these animals were collected on the same day. In adddition, blood serum and fresh segments of various regions of the brain (i.e. cerebellum, hypothalamus, thalamus, cerebral cortex), adenohypophysis and liver were collected. For Western blot and RT-PCR analyses, the specimens were immediately frozen on dry ice and stored at −80°C. For immunohistochemical studies, the specimens were immediately fixed.

Immunoprecipitation and Western blot analysis

Frozen tissues were homogenised in a homogenisation buffer (50 mM TRIS-HCl pH 7.0, 150 mM NaCl, 2 % Triton, 5 mM EDTA, 10 mg/ml leupeptin, 0.1 U/ml aprotinin, 1 mM phenylmethane sulfonyl-fluoride) by using an Ultra-Turrax homogeniser and centrifuged at 16,000*g* for 20 min at 4°C. The resulting supernatants were characterised for protein concentration by Bio-Rad protein assay. Equal amounts of proteins were immunoprecipitated overnight at 4°C with anti-UCN, -CRHR1 and –CRH2 (1 μg antibody/200 μg protein) previously bound to protein A/G-Agarose. A fraction of supernatant was used as an immunoprecipitation input control (as a loading control). Beads were sedimented by brief centrifugation and washed extensively with an ice-cold homogenisation buffer. Proteins, solubilised in a boiling sodium dodecyl sulphate (SDS) sample buffer (2% SDS, 5%L-mercaptoethanol, 66 mM TRIS pH 7.5, 10 mM EDTA), were separated on 18% and 12% SDS-polyacrylamide gels (Bio-Rad, Hercules, Calif., USA). In addition, aliquots of homogenate were subjected to electrophoresis on a 12% SDS-polyacrylamide gel. After electrophoresis, the gel was transferred to nitrocellulose by using a semi-dry apparatus (Bio-Rad) according to the manufacturer's instructions. The membrane was incubated for 1 h at 42°C in 5% bovine serum albumin (BSA; Sigma, St. Louis, Mo., USA) in TBST (150 mM NaCl, 20 mM TRIS-HCl pH 7.4, 0.3 % Tween-20), washed with TBST and incubated for 2 h at room temperature in the antisera diluted 1:500 in TBST containing 1% BSA. The following antisera were used: polyclonal goat anti-UCN (sc-1825; Santa Cruz Biotechnology,

Santa Cruz, Calif., USA), anti-CRHR1 (sc-12383; Santa Cruz Biotechnology), anti-CRHR2 (sc-1826; Santa Cruz Biotechnology), rabbit anti-CRHBP (SAB1300862; Sigma-Aldrich, St. Louis, Mo., USA) and mouse monoclonal anti-actin (JLA20 CP01; Calbiochem, San Diego, Calif., USA).

The membrane was washed three times with TBST, incubated for 1 h with anti-goat or anti-rabbit or anti-mouse IgG peroxidase conjugate (Vector Laboratories, Burlingame, Calif., USA) diluted 1:2000 in TBST-1% BSA and washed three times with TBST. Proteins were visualised by an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK). Marker proteins (coloured protein molecular weight markers; Prosieve, Lonza) were used to estimate the molecular weight of each band.

Immunohistochemistry

Fresh segments of tissues were fixed by immersion in Bouin's fixative (6–24 h), processed for paraffin embedding in a vacuum and cut at a thickness of 3–6 mμ. The avidin–biotin–peroxidase complex (ABC) method was performed by using the Vectastain ABC kit (Vector Laboratories) as described more fully elsewhere (Squillacioti et al. 2011). Primary antibodies were: polyclonal goat anti-UCN (diluted 1:500; sc-1825; Santa Cruz Biotechnology), anti-CRHR1 (diluted 1:500; sc-12383; Santa Cruz Biotechnology), anti-CRHR2 (diluted 1:500; sc-1826; Santa Cruz Biotechnology), rabbit anti-CRHBP (diluted 1:500; SAB1300862) and goat polyclonal anti-chromogranin A (diluted 1:1000; sc-1488; Santa Cruz Biotechnology).

The secondary antibodies were: biotinylated anti-goat or anti-rabbit IgG (diluted 1:200; Vector). The specificity of the immunoreactions was tested by replacing the primary antibody with a buffer. No immunoreaction was detected in control tests. The specificity of the primary antibodies was tested by pre-absorption with excess homologous antigen peptide and by using dot-blot assay as described more fully elsewhere (Squillacioti et al. 2011). The slides were observed by using a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany).

RNA extraction, cDNA synthesis, RT-PCR and sequencing

Samples of the frozen thyroid tissue were individually homogenised in ice-cold TRI-Reagent (Sigma) by using an Ultra-Turrax homogeniser. After chloroform extraction and isopropyl alcohol precipitation, RNA was dissolved in RNAase-free diethylpyrocarbonate-containing water. Total RNA was measured with an Eppendorf Biophotometer (Eppendorf, Basel, Switzerland). For cDNA synthesis, 1 μg total RNA was retro-transcribed with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA) and random hexamers as primers. For PCRs, specific primers for Equine UCN, CRH-R1, CRH-R2 and CRHBP were designed from the published gene sequences (*Equus caballus* UCN-like mRNA, Genbank accession number XM_001502361; *Equus caballus* CRHR1 mRNA, Genbank accession number XM_001496135, *Equus caballus* CRHR2 mRNA, Genbank accession number XM_001916709 and *Equus caballus* CRHBP mRNA, Genbank accession number XM_001918250) by using Primer Express software (PE Applied Biosystems).

The sense and anti-sense UCN primers used were 5'-CCGAGTCTGCGCTGGATT–3' and 5'-GTGGAAGGT GAGGTCAATGGA –3',respectively, which amplify a 177-bp fragment. The sense and anti-sense CRH-R1 primers were 5'-TACTACGACAACGAGAAGTGCTGGTT–' and 5'-AGCACCAGAGTGGCCTTCAC–3', respectively, which amplify a 200-bp fragment. The sense and anti-sense CRHR2 primers were 5'-ACGAAGTGCACGAGAGCAAC–3' and 5'-CCAGCACTGTTCGTTC–3', respectively, which amplify a 230-bp fragment. The sense and anti-sense CRHBP primers were 5'-CAGACCCCAAACGGAAGGTT-3' and 5'-CAGGGCCACGAAAGGGATA-3' respectively, which amplify a 250-bp fragment. In order to verify the efficiency of the RT and to exclude genomic DNA contamination, a fragment of β-actin cDNA (GenBank accession number NC_009156) was amplified and sequenced with primers designed to span an intron; the sense and anti-sense β-actin primers were 5'-CGAGGCCCAGAGCAAGAG-3' and 5'-GACATAGCAGAGCTTCTCCTTGATG-3'.

The PCR cycle conditions were as follows: 94°C (30 s), 60°C (30 s), 72°C (1 min) for 35 cycles; 72°C (5 min). A sample without cDNA template was used to verify that the master mix was free from contaminants. PCR products were electrophoresed on a 1.5% agarose gel and visualised under UV light. The PCR products of equine UCN, CRHR1 and CRHR2 were purified by using a GFX PCR DNA and Gel Purification Kit (code 28-9034-70, GE Healthcare) and sequenced.

Results

Immunoprecipitation and Western blot

The results of the immunoprecipitation and Western blot analysis are shown in Fig. 1. Tissue extracts of the thyroid gland of the horse reacted with the anti-UCN, -CRHR1 and –CRHR2 antibodies.

Thyroid tissue extracts and the synthetic peptide UCN reacted with the anti-UCN antibody (Fig. 1). The antibody recognised one major protein band of approximately 16 kDa from homogenates and one protein band of approximately 6.5 kDa from the synthetic peptide.

The thyroid tissue extracts also reacted with the anti-CRHR1 and anti-CRHR2 antibodies (Fig. 1). The antibodies

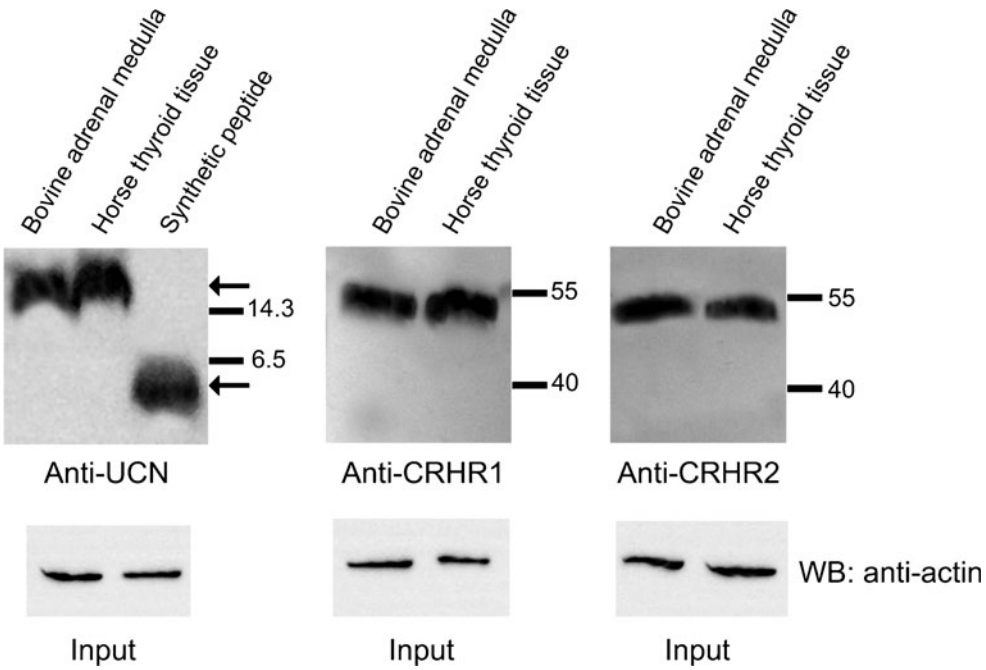

Fig. 1 Expression of urocortin (*UCN*), corticotropin-releasing hormone (CRH) type 1 receptor (*CRHR1*) and CRH type 2 receptor (*CRHR2*) by Western blot (*WB*) in the horse thyroid gland. Tissue extracts were immunoprecipitated and immunoblotted with anti-UCN, -CRHR1 and –CRHR2 antibodies; UCN synthetic peptide (100 ng/lane) and bovine adrenal medulla were used as positive controls. Beta-actin acted as the input loading control of immunoprecipitated proteins. Molecular weight markers are expressed in kDa

recognised one protein band measuring approximately 55 kDa. The thyroid tissue, in addition, did not react with the anti-CRHBP antibody (Fig. 2). As a CRHBP-positive control, we used horse adenohypophysis since CRHBP is known to be highly expressed in this gland (Potter et al. 1992) in which it is located in adrenocorticotroph cells. In addition, CRHBP antibody reacted with cerebellum, cerebral cortex, thalamus and hypothalamus extracts. The antibody recognised one protein band measuring approximately 37 kDa. Serum and liver did not react (Fig. 2).

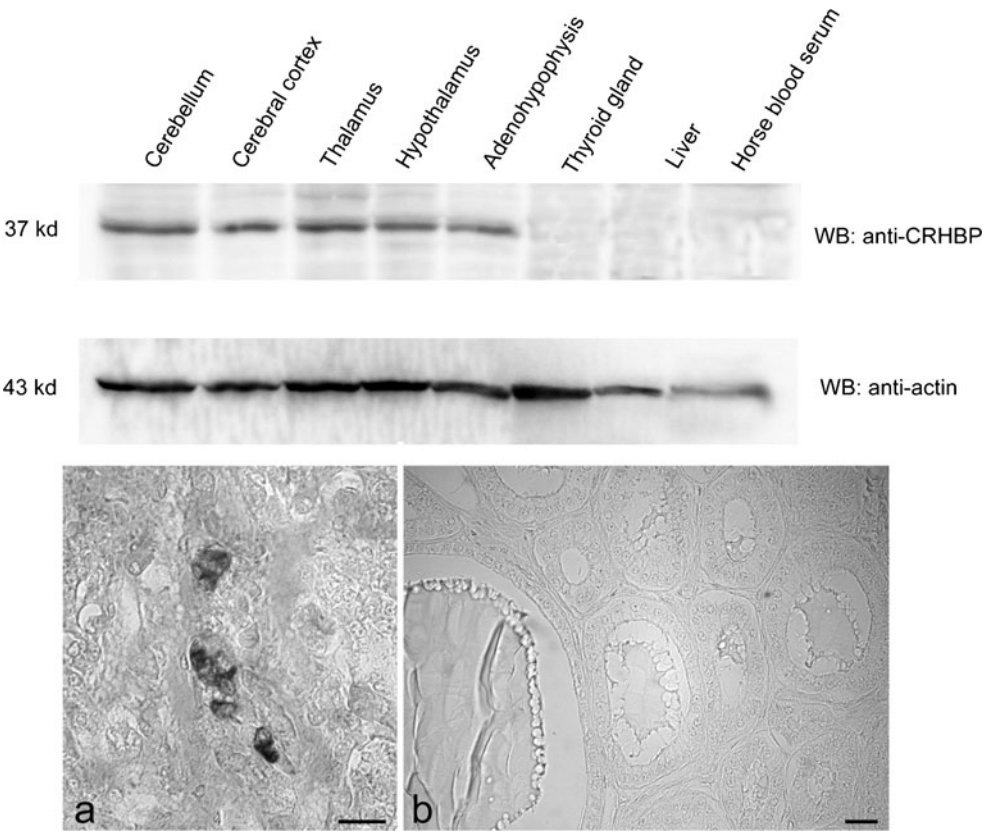

Fig. 2 CRH-binding protein (*CRHBP*) expression by Western blot (*WB*) and immunohistochemistry in the horse thyroid gland. CRHBP immunoreactivity (-IR) was not detected in tissue extracts of thyroid gland, liver or horse blood serum (*top right*) or in the tissue sections of the thyroid gland (**b**). In the positive controls, CRHBP-IR was detected in the tissue extracts of the adenohypophysis (positive control), various regions of the brain (*top left*) and in the cells of the adrenocorticotroph cells (**a**). The blot was stripped and incubated with anti-actin antibody as a loading control (*middle*)

Immunohistochemistry

In the horse thyroid gland, UCN-IR was found in the follicular cells (Fig. 3a, d). CRHR2-IR was detected in the C-cells (Fig. 3c, g). As shown by chromogranin A immunohistochemistry, C-cells are larger than follicular cells and have a large rounded nucleus. They usually do not extend to the lumen of the follicles (Fig. 3f). Alternate CRHR2 and chromogranin A antibody staining on serial 3-μm-thick sections demonstrated a colocalisation of these two antigens in C-cells.

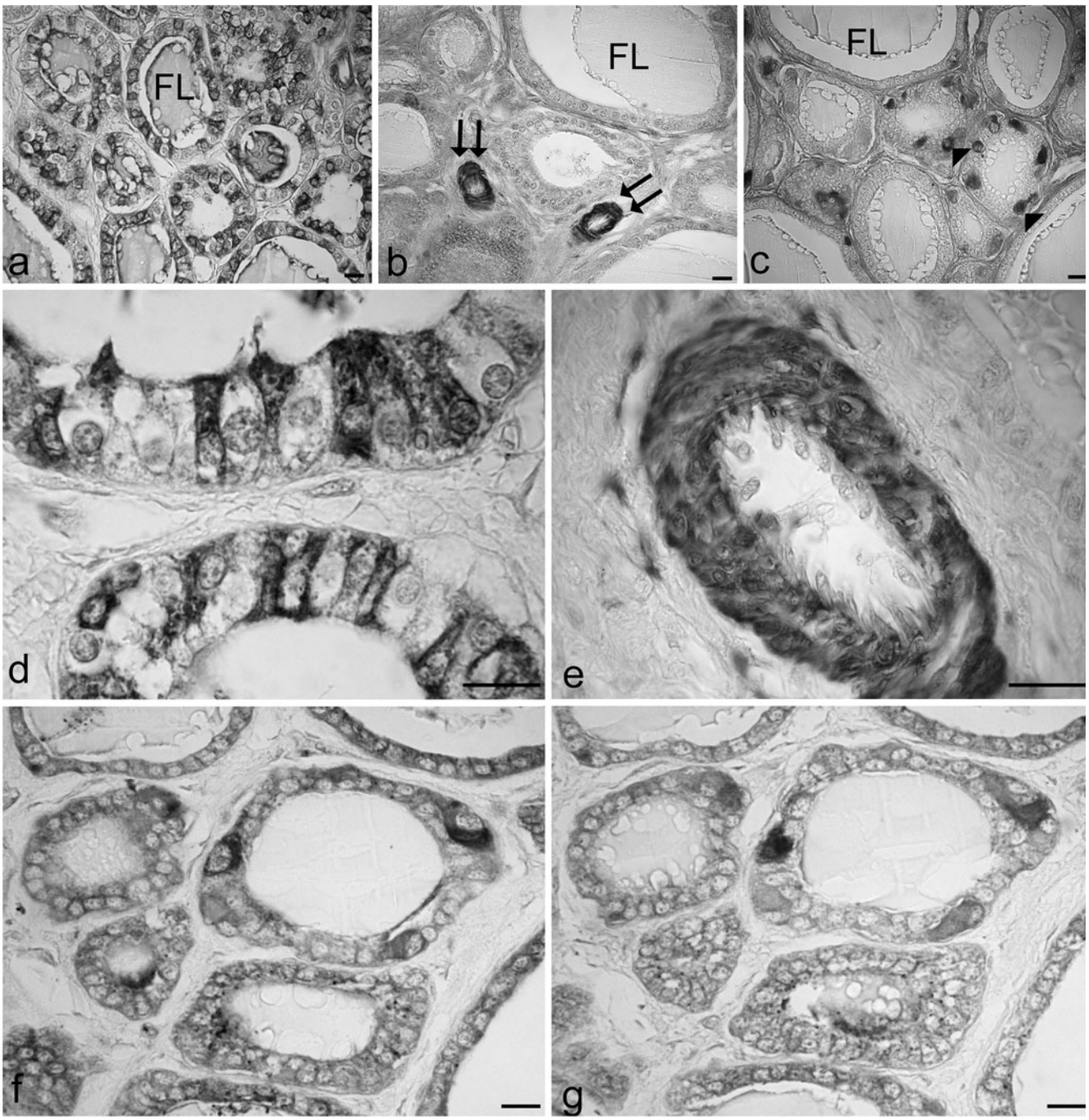

Fig. 3 Distribution of the UCN-, CRHR1- and CRHR2-immunoreactivities in the horse thyroid gland. UCN-IR was distributed in thyroid follicular cells (**a**). Higher magnification shows UCN-immunoreactive epithelial cells (**d**). CRHR1-IR was located in the blood vessels (**b**). Higher magnification of the thyroid blood vessel shows CRHR1-immunoreactive cells in the vascular smooth muscle (**e**). CRHR2–IR was distributed in the C-cells (**c**, **g**) as demonstrated by staining with anti-chromogranin A antibody (as a marker of C-cells) of the previous 3-μm-thick serial section (**f**). The *double arrows* indicate CRHR1-positive vascular smooth muscle cells (*arrowheads* positive C-cells, *FL* follicular lumen, *L* blood vessel lumen). *Bar* 10 μm

The distribution of CRHR1-IR was different from that for UCN and CRHR2. CRHR1-IR was found only in the smooth musculature of blood vessels (Fig. 3b, e).

CRHBP-IR was not detected in the horse thyroid gland (Fig. 2b). In IR controls, CRBP-IR was detected in the cells of the adrenocorticotroph cells (Fig. 2a).

RT-PCR analysis

RT-PCR experiments demonstrated that UCN, CRHR1 and CRHR2 mRNAs were expressed in the thyroid gland, whereas CRHBP mRNA was not expressed (Fig. 4). However, CRHBP mRNA was expressed in the cerebellum, cerebral cortex, hypothalamus, thalamus and adenohypophis (Fig. 4).

Discussion

The results of the present study revealed the presence of UCN, CRHR1, CRHR2 mRNA and protein in the thyroid gland of a mammalian species by using various experimental approaches. Specifically, immunoprecipitation and Western blot analysis were used to study the presence of UCN and its receptors. Immunohistochemical studies were performed to evaluate the distribution and localisation of these proteins, whereas RT-PCR experiments were performed to reveal their mRNA. RT-PCR experiments revealed that UCN, CRHR1 and CRHR2 mRNAs were expressed in the horse thyroid gland, whereas CRHBP was not expressed, confirming the immunohistochemical staining data.

Up until now, the expression of CRH-related peptides has only been reported in the human thyroid gland under pathological conditions. CRH, UCN 1 and UCN 3 have been found to be expressed in cases of multiple endocrine neoplasia type II accompanied by thyroid medullary carcinoma and phaeochromocytomas (Kageyama et al. 2008). In addition, CRH-IR has been detected in thyroid lesions, predominantly in those related to autoimmune phenomena (Scopa et al. 1994). Thyroid CRH might act as a proinflammatory cytokine during the inflammatory processes accompanying thyroid disorders.

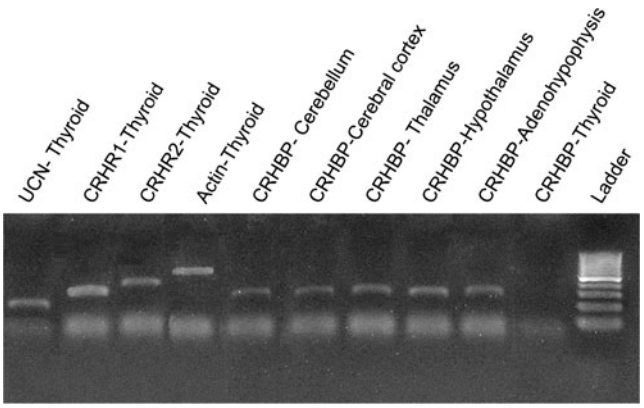

Fig. 4 UCN, CRHR1, CRHR2 and CRHBP expression as shown by reverse transcription with the polymerase chain reaction in the horse thyroid gland. UCN (177-bp fragment), CRH-R1 (200-bp fragment) and CRH-R2 (230-bp fragment) were expressed in the horse thyroid; β-actin (490-bp fragment) acted as a positive control. CRHBP (230-bp fragment) was not expressed in the horse thyroid, whereas it was expressed in the adenohypophysis, cerebellum, cerebral cortex, thalamus and hypothalamus (*M* molecular markers of 100-bp ladder)

In non-mammalian vertebrates, CRH appears to be a potent stimulator of hypophyseal thyroid-stimulating hormone (TSH) secretion and might therefore function as a common regulator of both the thyroidal and adrenal/interrenal axes (De Groef et al. 2006; Kühn et al. 1998). CRH exerts its dual hypophysiotropic action through two different types of CRHRs (De Groef et al. 2003a, 2003b). Thyrotropes express type 2 CRHRs, while CRH-induced corticotropin (ACTH) secretion is mediated by type 1 CRHRs on the corticotropic pituitary cells.

No data are available regarding the expression of UCN and CRHR mRNAs in normal mammalian thyroid tissue. Previous studies have demonstrated the presence of a UCN-ergic system under normal conditions in other endocrine glands, i.e. adrenal gland (Squillacioti et al. 2011; Dermitzaki et al. 2007), ovary (Muramatsu et al. 2001) and testis (Lee et al. 2011).

Immunoprecipitation and Western blot analysis have shown that the anti-mammalian UCN antibody used in the present study recognises a protein band with a molecular weight of approximately 16 kDa in the horse thyroid gland. This protein is comparable with the mammalian UCN precursor, which is a 122-amino-acid protein (Vaughan et al. 1995; Donaldson et al. 1996; Squillacioti et al. 2011). The antibodies used against CRHR1 and CRHR2 recognise protein bands with molecular weights of approximately 55 kDa. These proteins are comparable with the mammalian CRHRs (Perrin et al. 2006; Chen et al. 1993). The antibody anti-CRHBP does not recognise any protein bands in the horse thyroid tissue or in the horse liver and serum, while recognising a protein band in the adenohypophysis and various brain regions. CRHBP has been found to be expressed in the brain and pituitary gland of several mammalian species (Potter et al. 1992; Baigent and Lowry 2000; Cortright et al. 1995; Behan et al. 1996; Peto et al. 1999) and in human blood serum (Orth and Mount 1987), whereas it has been reported to be absent from rodent and equine serum (Potter et al. 1991, 1992; Ellis et al. 1994). CRHBP is an important modulator of CRH or UCN activity. In the absence of CRHBP, CRH and other CRH-like ligands are no longer bound, leading to increased CRH/UCN bioactivity. Our results suggest that CRHBP in the horse plays a role in antagonising the effects of CRH-related peptides in the brain and pituitary gland but not in the thyroid gland.

The results of the present study have shown that, in the horse thyroid gland, UCN-IR is present in the follicular

cells, whereas CRHR2-IR occurs in the C-cells. Thus, UCN might affect calcitonin secretion via CRHR2 in a paracrine manner. Additionally, other CRHR2 ligands, such as UCN2 and UCN3, probably interact with this receptor to regulate C-cell activity.

C-cells are primarily known for producing calcitonin, a hypocalcaemic and hypophosphataemic hormone. Nevertheless, in addition to their role in calcium homeostasis, C-cells might be involved in the intrathyroidal regulation of follicular cells, further suggesting a possible interrelationship between the two endocrine populations. C-cells produce and secrete a number of different regulatory peptides. Some of these regulatory peptides display an inhibiting action on thyroid hormone secretion, such as calcitonin, calcitonin gene-related peptide and somatostatin (Ahren 1989, 1991; Zerek-Melen et al. 1989), whereas others act as local stimulators of thyroid hormone synthesis, such as gastrin-releasing peptide, helodermin and serotonin (Ahren 1989; Grunditz et al. 1989; Tamir et al. 1992). Despite the receptors for some of the above mentioned substances, e.g. somatostatin or serotonin, being expressed by follicular cells (Tamir et al. 1992, 1996; Ain et al. 1997), a clear role has not yet been assigned to these C-cell-secreted regulatory factors.

Furthermore, ghrelin and thyrotropin-releasing hormone, the two additional regulatory peptides that orchestrate the hypothalamic control of the thyroid function through thyrotropin, are also expressed in C-cells (Gkonos et al. 1989; Korbonits et al. 2001; Raghay et al. 2006). Rat and human C-cell lines express the thyrotropin receptor at both mRNA and protein levels. These results confirm that C-cells, under the regulation by thyrotropin, are involved in the hypothalamic-pituitary-thyroid axis and suggest a putative role in the local fine-tuning of follicular cell activity (Morillo-Bernal et al. 2009).

Recently, additional evidence has been presented that a functional interaction between follicular and C-cells mediated by the release of the peptidergic hormones or neuropetides does indeed exist (Morillo-Bernal et al. 2009, 2011).

As far as we know, no data concerning the regulation of C-cells by follicular cells have been published. However, this kind of intrathyroidal regulation has recently been postulated, as evidence has become available that follicular and C-cells evolve with the same rhythm during the hyperstimulation or suppression of thyroid function (Martín-Lacave et al. 2009).

We have found CRHR1-IR in the vascular smooth musculature, thus suggesting a role for UCN in modulating the blood flow of the thyroid gland. This hypothesis is corroborated by the finding that UCN is a dilator of rat (Abdelrahman et al. 2005; Lubomirov et al. 2001) and mouse (Lubomirov et al. 2006) arteries.

Thyroid blood flow together with thyrotropin (TSH) regulates the uptake of iodide by the thyroid gland (Arntzenius et al. 1991; Ingbar et al. 1986). Iodide is an essential element for the production of thyroid hormones. Alterations in the thyroidal uptake of iodide can cause changes in the production of thyroid hormones and the regulation of iodide uptake is important for thyroid gland activity.

In conclusion, these results suggest that UCN, CRHR1 and CRHR2 are expressed in the horse thyroid gland and that a local regulatory system of thyroid functions based on UCN and CRHRs exists. UCN plays a role in the regulation of the blood flow of this gland via CRHR1 and of calcitonin synthesis via CRHR2. In addition, the bioavailability of the UCN in this system is not modulated by CRHBP, because this protein is not expressed in the horse thyroid gland.

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