ANATOMIA HISTOLOGIA EMBRYOLOGIA
JOURNAL OF VETERINARY MEDICINE
Anatomia, Histologia, Embryologia

ORIGINAL ARTICLE

Presence and Distribution of Urocortin and Corticotrophin-Releasing Hormone Receptors in the Bovine Thyroid Gland

C. Squillacioti*, A. De Luca, S. Alì, R. Ciarcia, G. Germano, A. Vittoria and N. Mirabella

Address of authors: Dipartimento di Medicina Veterinaria e Produzioni animali, Università di Napoli "Federico II", Via Federico Delpino, 1-80137 Napoli, Italia

***Correspondence:**
Tel.: +390812536113;
fax: +390812536097;
e-mail: caterina.squillacioti@unina.it

With 3 figures

Received August 2013; accepted for publication October 2013

doi: 10.1111/ahe.12094

Summary

Urocortin (UCN), a 40 amino acid peptide, is a corticotrophin-releasing hormone (CRH)-related peptide. The biological actions of CRH family peptides are mediated via two types of G-protein-coupled receptors, CRH type 1 (CRHR1) and CRH type 2 (CRHR2). The aim of this study was to investigate the expression of UCN, CRHR1 and CRHR2 by immunoprecipitation, Western blot, immunohistochemistry and RT-PCR in the bovine thyroid gland. Immunoprecipitation and Western blot analysis showed that tissue extracts reacted with the anti-UCN, anti-CRHR1 and anti-CRHR2 antibodies. RT-PCR experiments demonstrated that mRNAs of UCN, CRHR1 and CRHR2 were expressed. UCN immunoreactivity (IR) and CRHR2–IR were found in the thyroid follicular and parafollicular cells and CRHR1-IR in the smooth muscle of the blood vessels. These results suggest that a regulatory system exists in the bovine thyroid gland based on UCN, CRHR1 and CRHR2 and that UCN plays a role in the regulation of thyroid physiological functions through an autocrine/paracrine mechanism.

Introduction

Urocortin (UCN), a 40-amino acid peptide originally cloned from the Edinger–Westphal nucleus, is a member of the corticotrophin-releasing hormone (CRH) family of peptides (Vaughan et al., 1995). In addition to CRH and UCN, the CRH family includes the more recently identified peptides, Urocortin 2 (UCN2) and Urocortin 3 (UCN3) (Lewis et al., 2001; Reyes et al., 2001). The effects of CRH and UCNs are mediated by the distinct G-coupled receptors, termed CRH receptor type 1 (CRHR1) and 2 (CRHR2) (Perrin et al., 1993, 2006). These receptors are encoded by two separate genes and significantly differ in pharmacological profile, tissue distribution and function, although they display 70% homology in amino acid sequence. CRH has a very high affinity for CRHR1. UCN binds with the same affinity to both receptors, and UCN2 and UCN3 have high affinity to CRHR2 (Tu et al., 2007). In addition to the receptors, there is a CRH-binding protein (CRH-BP), which has been reported in mammalian and non-mammalian vertebrates (Behan et al.,1989; Potter et al., 1992; Cortright et al.,1995; Seasholtz et al., 2002). UCN and CRHRs gene expression and/or immunoreactivity have been detected in several peripheral organs belonging to digestive, cardiovascular, immune and reproductive systems (Oki and Sasano, 2004; De Luca et al., 2009; Yang et al., 2010; Lee et al., 2011).

The presence of UCN and its receptors was, moreover, reported in several mammalian (Tsatsanis et al., 2007; Huising et al., 2010; Squillacioti et al., 2011, 2012) endocrine glands, thus suggesting a role for this peptide in regulating endocrine functions.

To further highlight the role of urocortin in the control of thyroid function, we investigate in this study the expression and distribution of UCN, CRHR1 and CRHR2 in the bovine thyroid gland. For this purpose, we performed immunoprecipitation, Western blot and immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR).

Materials and Methods

Animals and tissue collection

This study was performed using adult healthy animals coming from the same farm located in Southern Italy.

The animals were bred in natural environmental conditions and were fed hay and concentrate. Water was available continuously. The animals were sacrificed in a public slaughterhouse according to the current Italian animal care protocols. Bovine thyroid and adrenal glands from these animals were collected in the same day. For Western blot and RT-PCR analyses, fresh segments of thyroid gland and adrenal medulla (positive control) were immediately frozen on dry ice and stored at −80°C. For immunohistochemical studies, fresh segments of thyroid gland were immediately fixed.

Immunoprecipitation and Western blot analysis

Frozen tissues were homogenised in a buffer (50 mM Tris–HCl pH 7.00; 150 mM NaCl; 2% Triton; 5 mM EDTA; 10 μg/ml leupeptin; 0.1 U/ml aprotinin; 1 mM PMSF) 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, anti-CRHR1 and anti-CRHR2 anti-sera (1 μg antibody/200 μg proteins) 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 ice-cold homogenisation buffer. Proteins, solubilised in 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, CA, western USA). After electrophoresis, the gel was transferred to nitrocellulose using a semi-dry apparatus (Bio-Rad, Hercules, CA, USA) 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 (RT) in the anti-sera diluted 1:500 in TBST containing 1% BSA. The following primary anti-sera were used: polyclonal goat anti-UCN (sc-1825, diluted 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-CRHR1 (sc-12383, diluted 1:1000; Santa Cruz Biotechnology) and anti-CRHR2 (sc-1826, diluted 1:1000; Santa Cruz Biotechnology). The membrane was washed three times with TBST, incubated for 1 h with anti-goat IgG conjugated to peroxidase (Vector Laboratories, Burlingame, CA, USA) diluted 1:2000 in 1% BSA containing TBST 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. β-Actin acted as the input loading control of immunoprecipitated proteins. A fraction of supernatant (50 μg) was separated on SDS-PAGE and immunoblotted with an anti-β-actin primary antibody (Mouse mAb JLA20 cp 01, diluted 1:5000; Calbiochem, San Diego, CA, USA) and, successively, with a peroxidase-conjugated rabbit anti-mouse IgG secondary antibody (diluted 1: 2000; Vector Laboratories).

Immunohistochemistry

Fresh fragments of thyroid gland 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 μm. The avidin–biotin–peroxidase complex (ABC) method was performed using the Vectastain ABC kit (Vector Laboratories) as described more fully elsewhere (Squillacioti et al., 2011). Primary anti-sera were the same described in detail in the precedent section and were directed against UCN, CRHR1 and CRHR2 (diluted 1:200). The secondary antibodies were as follows: biotinylated anti-goat IgG (diluted 1:200; Vector Laboratories). The specificity of the primary immunoreactions was tested by replacing each antibody with a buffer or preabsorbing the antibody with an excess (100 μg antigen/ml anti-serum as the final dilution) of the relative antigen or, finally, using a dot-blot assay as described more fully elsewhere (Squillacioti et al., 2011). No immunoreaction was detected in control tests. The slides were observed 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) using an Ultra-Turrax homogeniser. After chloroform extraction and isopropyl alcohol precipitation, RNA was dissolved in RNase-free DEPC water. Total RNA was measured with an Eppendorf Biophotometer (Eppendorf AG, Basel, Switzerland). For cDNA synthesis, 1 μg of total RNA was retrotranscribed with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) and random hexamers as primers. For PCRs, specific primers for bovine UCN, CRH-R1 and CRH-R2 were designed from the published gene sequences using the Primer Express™ software (PE Applied Biosystems) as described elsewhere (Squillacioti et al., 2011). To verify the efficiency of the reverse transcription (RT) and to exclude genomic DNA contamination, a fragment of β-actin cDNA (GenBank accession number NM_173979)

was amplified and sequenced with primers designed to span an intron. The sense and anti-sense beta-actin primers were 5′-CAG CTC CTC CCT GGA GAA GA-3′ and 5′-CTG CTT GCT GAT CCA CAT CTG-3′, which amplify a 398-bp fragment. 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 bovine UCN, CRHR1, CRHR2 were purified using 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 bovine thyroid gland reacted with the anti-UCN, anti-CRHR1 and anti-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 recognised one protein band measuring approximately 55 kDa.

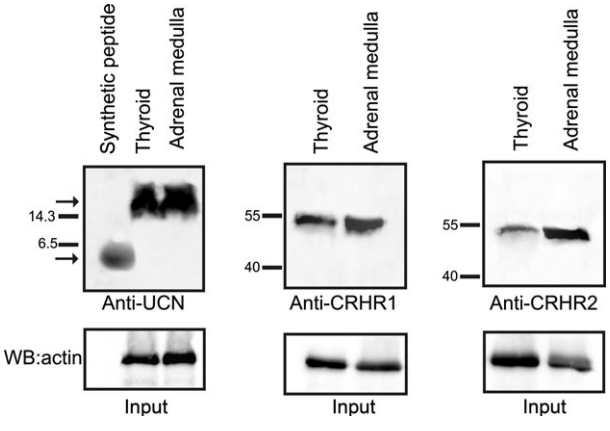

Fig. 1. *Expression of UCN, CRHR1 and CRHR2 by immunoprecipitation and Western blot analysis in the bovine thyroid gland*. Tissue extracts were immunoprecipitated and immunoblotted with anti-UCN, anti-CRHR1 and anti-CRHR2 antibodies; UCN synthetic peptide (100 ng/lane) and bovine adrenal medulla were used as positive controls. Beta-actin acted as the input loading control of immunoprecipitated proteins. Molecular weight markers are in kDa.

Immunohistochemistry

In the bovine thyroid gland, UCN immunoreactivity (IR) was found in follicular (Fig. 2a) and parafollicular cells (C cells) (Fig. 2b). In the follicular cells, the immunopositive material had a supranuclear distribution. CRHR2-IR was also found in the follicular and parafollicular cells (Figs 2c and 3d). Parafollicular cells were larger than follicular and had large rounded nuclei. They usually did not reach the lumen of the follicles (Fig 2b,d). The distribution of CRHR1-IR was different from UCN and CRHR2. CRHR1-IR was found only in the smooth musculature of blood vessels (Fig. 2e,f).

RT-PCR

RT-PCR experiments demonstrated that UCN, CRHR1 and CRHR2 mRNAs were expressed in the thyroid gland (Fig. 3).

Discussion

The results of the present study revealed the presence of UCN, CRHR1 and CRHR2 mRNAs and proteins in the bovine thyroid gland.

Up until now, the expression of UCN and CRHRs in non-pathological thyroid tissue has only been reported in the horse (Squillacioti et al., 2012). CRH, UCN and UCN3 have been also reported in the human thyroid gland in cases of multiple endocrine neoplasia type II accompanied by thyroid medullary carcinoma and phaeochromocytomas (Kageyama et al., 2008). CRH-IR, moreover, has been detected in thyroid lesions, predominantly in those related to autoimmune phenomena (Scopa et al., 1994).

Immunoprecipitation and Western blot analysis showed that the anti-mammalian UCN antibody used in the present study recognised a protein band with a molecular weight of approximately 16 kDa in the bovine 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 recognised protein bands with molecular weights of approximately 55 kDa. These proteins are comparable with the mammalian CRH receptors (Chen et al., 1993; Perrin et al., 2006).

The results of the present study showed that in the bovine thyroid gland, UCN- and CRHR2-IRs were found in the follicular and parafollicular cells. These results differ from those reported in the horse thyroid gland where UCN-IR was found in follicular and CRHR2-IR in parafollicular cells (Squillacioti et al., 2012). UCN may play

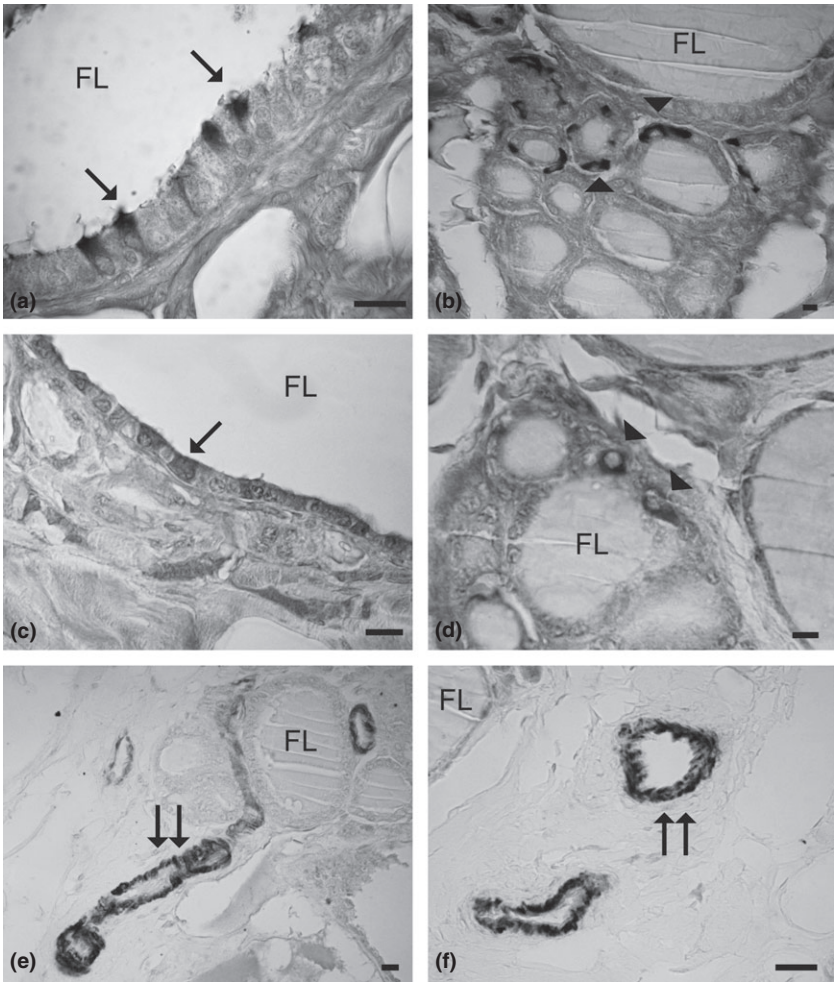

Fig. 2. *Distribution of the UCN, CRHR1 and CRHR2 immunoreactivities in the bovine thyroid gland.* UCN-IR was distributed in the apical portion of the thyroid follicular cells (a) (arrows) and in the thyroid parafollicular cells (b) (arrow head). CRHR2-IR was distributed in the thyroid follicular (c) and parafollicular cells (d). CRHR1-IR was found in the vascular smooth muscle (e, f) (double arrows) of the thyroid blood vessels. FL Follicular lumen bar = 25 micron.

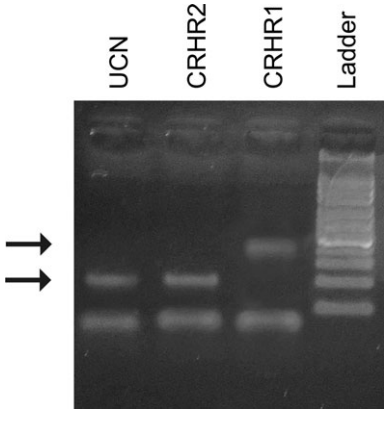

Fig. 3. *UCN, CRHR1 and CRHR2 expression by RT-PCR in the bovine thyroid gland.* UCN (200-bp fragment), (2) CRH-R2 (200-bp fragment), (3) CRH-R1 (409-bp fragment) were expressed in the bovine thyroid gland. Molecular weight marker was a 100-bp ladder.

some roles in the regulation of thyroid functions such as Ca^{2+} metabolism and hormone secretion. Besides the extrathyroidal main regulation, thyroid functions and growth are under the influence of intrathyroidal peptides. There is evidence of the role played by some local factors released by nerve endings, such as vasointestinal peptide (VIP) or neuropeptide Y (Ahren, 1989), by follicular cells themselves, such as insulin-like growth factors (IGFs) (review, Eggo et al., 2003) or GHrelin (Morillo-Bernal et al., 2011), and by parafollicular cells such as serotonin and calcitonin gene-related peptide (CGRP) (Ahren, 1991; Utrilla et al., 2013). Functional interaction between follicular and parafollicular cells mediated by the release of the peptidergic hormones or neuropeptides seems to exist (Bernal et al., 2009; Morillo-Bernal et al., 2011). Thyroid follicular cells are responsible for the thyroglobulin synthesis, iodine uptake and thyroid hormone synthesis. The production and secretion of thyroxine and triiodothyronine by the thyroid gland are stimulated by the hypothalamic hormone thyrotropin-releasing hormone (TRH) and the anterior pituitary hormone TSH. Thyroid parafollicular cells are mainly known for producing calcitonin, a hormone involved in calcium homeostasis with hypocalcaemic and hypophosphataemic effects.

Our results suggest that UCN could affect thyroid hormones and calcitonin secretion via CRHR2 in an autocrine/paracrine manner. In addition, other CRHR2 ligands, such as UCN2 and UCN3, probably interact with this receptor to regulate follicular and parafollicular cell activity.

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

The thyroid blood flow together with TSH regulates the uptake of iodide by the thyroid gland (Ingbar et al., 1986; Arntzenius et al., 1991). 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 the iodide uptake is very important for the thyroid gland activity.

In conclusion, these results suggest that UCN, CRHR1 and CRHR2 are expressed in the bovine thyroid gland and that a local regulatory system of thyroid functions based on the UCN and CRHRs exists. UCN plays a role in the regulation of the blood flow of this gland via CRHR1 and of the thyroid hormones synthesis via CRHR2 by an autocrine/paracrine mechanism.

References

Abdelrahman, M., H. T. Syyong, A. A. Tjahjadi, and C. C. Pang, 2005: Analysis of the mechanism of the vasodepressor effect of urocortin in anesthetized rats. Pharmacology **7**, 175–179.

Ahren, B., 1989: Effects of calcitonin, katacalcin, and calcitonin gene related peptide on basal and TSH-stimulated thyroid hormone secretion in the mouse. Acta Physiol. Scand. **135**, 133–137.

Ahren, B., 1991: Regulatory peptides in the thyroid gland – a review on their localization and function. Acta. Endocrinol. (Copenh) **124**, 225–232.

Arntzenius, A. B., L. J. Smit, J. Schipper, D. Heide, and A. E. Meinders, 1991: Inverse relation between iodine intake and thyroid blood flow: color Doppler flow imaging in euthyroid humans. J. Clin. Endocrinol. Metab. **75**, 1051–1055.

Behan, D. P., E. A. Linton, and P. J. Lowry, 1989: Isolation of the human plasma corticotrophin-releasing factor-binding protein. J. Endocrinol. **122**, 23–31.

Bernal, J. M., J. M. Fernández-Santos, J. C. Utrilla, M. de Miguel, R. García-Marín, and I. Martín-Lacave, 2009: Functional expression of the thyrotropin receptor in C cells: new insights into their involvement in the hypothalamic-pituitary-thyroid axis. J. Anat. **215**, 150–158.

Chen, R., K. A. Lewis, M. H. Perrin, and W. W. Vale, 1993: Expression cloning of a human corticotropin-releasing-factor receptor. Proc. Natl Acad. Sci. USA **90**, 8967–8971.

Cortright, D. N., A. Nicoletti, and A. F. Seasholtz, 1995: Molecular and biochemical characterization of the mouse brain corticotropin-releasing hormone-binding protein. Mol. Cell. Endocrinol. **111**, 147–157.

De Luca, A., C. Squillacioti, M. E. Pero, S. Paino, E. Langella, and N. Mirabella, 2009: Urocortin-like immunoreactivity in the primary lymphoid organs of the duck (*Anas platyrhynchos*). Eur. J. Histochem. **53**, 167–176.

Donaldson, C. J., S. W. Sutton, M. H. Perrin, A. Z. Corrigan, K. A. Lewis, and J. Rivier, 1996: Cloning and characterization of human urocortin. Endocrinology **137**, 3896.

Eggo, M. C., V. M. Quiney, and S. Campbell, 2003: Local factors regulating growth and function of human thyroid cells in vitro and in vivo. Mol. Cell. Endocrinol. **213**, 47–58.

Huising, M. O., T. van der Meulen, J. M. Vaughan, M. Matsumoto, C. J. Donaldson, H. Park, N. Billestrup, and W. W. Vale, 2010: CRFR1 is expressed on pancreatic beta cells, promotes beta cell proliferation, and potentiates insulin secretion in a glucose-dependent manner. PNAS **107**, 912–917.

Ingbar, S. H., L. E. Braverman, and A. Taurog, 1986: Hormone synthesis: thyroid iodine metabolism. In: The Thyroid. Section B: Hormone Synthesis and Secretion, 5th edn. (S. H. Ingbar and L. E. Braverman, eds). Philadelphia, PA: Lippincott. pp. 53–93.

Kageyama, K., S. Sakihara, M. Yamashita, K. Takahashi, S. Kawashima, J. Tanabe, S. Tsutaya, M. Yasujima, and T. Suda, 2008: A case of multiple endocrine neM. neoplasia type II accompanied by thyroid medullary carcinoma and pheochromocytomas expressing corticotropin-releasing factor and urocortins. Am. J. Med. Sci. **335**, 398–402.

Lee, S., B. Braden, S. S. Kang, and C. Rivier, 2011: Urocortins are present in the rat testis. Neuropeptides **45**, 131–137.

Lewis, K., C. Li, M. H. Perrin, A. Blount, K. Kunitabe, C. Donaldson, J. Vaughan, T. M. Reyes, J. Gulyas, W. Fischer, L. Bilezikjian, J. Rivier, P. E. Sawchenko, and W. W. Vale, 2001: Identification of urocortin III, an additional member of the corticotrophin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc. Natl Acad. Sci. USA **98**, 7570–7575.

Lubomirov, L. T., H. Gagov, P. Petkova-Kirova, D. Duridanova, V. U. Kalentchuk, and R. Schubert, 2001: Urocortin relaxes rat tail arteries by a PKA-mediated reduction of the sensitivity of the contractile apparatus for calcium. Br. J. Pharmacol. **134**, 1564–1570.

Lubomirov, L. T., K. Reimann, D. Metzler, V. Hasse, R. Stehle, M. Ito, D. J. Hartshorne, H. Gagov, G. Pfitzer, and R. Schubert, 2006: Urocortin-induced decrease in Ca^{2+} sensitivity of contraction in mouse tail arteries is attributable to cAMP-dependent dephosphorylation of MYPT1 and activation of myosin light chain phosphatase. Circ. Res. **98**, 1159–1167.

Morillo-Bernal, J. M., J. M. Fernández-Santos, M. Miguel, R. García-Marín, F. Gordillo-Martínez, E. Díaz-Parrado, J. C. Utrilla, and I. Martín-Lacave, 2011: Ghrelin potentiates TSH-induced expression of the thyroid tissue-specific genes thyroglobulin, thyroperoxidase and sodium-iodine symporter, in rat PC-Cl3 Cells. Peptides **32**, 2333–2339.

Oki, Y., and H. Sasano, 2004: Localization and physiological roles of urocortin. Peptides **25**, 1745–1749.

Perrin, M. H., C. J. Donaldson, R. Chen, K. A. Lewis, and W. W. Vale, 1993: Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology **133**, 3058–3061.

Perrin, M. H., C. R. Grace, R. Riek, and W. W. Vale, 2006: The three-dimensional structure of the N-terminal domain of corticotropin-releasing factor receptors: sushi domains and the B1 family of G protein-coupled receptors. Ann. N. Y. Acad. Sci. **1070**, 105–119.

Potter, E., D. P. Behan, E. A. Linton, P. J. Lowry, P. E. Sawchenko, and W. W. Vale, 1992: The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. Proc. Natl Acad. Sci. USA **419**, 2–6.

Reyes, T. M., K. Lewis, M. H. Perrin, K. S. Kunitake, J. Vaughan, C. A. Arias, J. B. Hogenesch, J. Gulyas, J. Rivier, W. W. Vale, and P. E. Sawchenko, 2001: Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc. Natl Acad. Sci. USA **98**, 2843–2848.

Scopa, C. D., G. Mastorakos, T. C. Friedman, M. Melachrinou, M. J. Merino, and G. P. Chrousos, 1994: Presence of immunoreactive corticotrophin releasing hormone in thyroid lesions. Am. J. Pathol. **145**, 1159–1167.

Seasholtz, A. F., R. A. Valverde, and R. J. Denver, 2002: Corticotropin-releasing hormone-binding protein: biochemistry and function from fishes to mammals. J. Endocrinol. **175**, 89–97.

Squillacioti, C., A. De Luca, G. Liguori, S. Paino, and N. Mirabella, 2011: Expression of urocortin and corticotropin-releasing hormone receptors in the bovine adrenal gland. Gen. Comp. Endocrinol. **172**, 416–422.

Squillacioti, C., A. De Luca, S. Alì, S. Paino, G. Liguori, and N. Mirabella, 2012: Expression of urocortin and corticotropin-releasing hormone receptors in the horse thyroid gland. Cell Tissue Res. **350**, 45–53.

Tsatsanis, C., E. Dermitzaki, M. Venihaki, E. Chatzaki, V. Minas, A. Gravanis, and A. Margioris, 2007: The corticotropin-releasing factor (CRF) family of peptides as local modulators of adrenal function. Cell. Mol. Life Sci. **64**, 1638–1655.

Tu, H., A. J. Kastin, C. Bjorbaek, and W. Pan, 2007: Urocortin trafficking in cerebral microvessel endothelial cells. J. Mol. Neurosci. **31**, 171–181.

Utrilla, J. C., J. Morillo-Bernal, F. Gordillo-Martínez, R. García-Marín, J. L. Herrera, J. M. Fernández-Santos, E. Díaz-Parrado, C. Garnacho, M. De Miguel, and I. Martín-Lacave, 2013: Expression of hypothalamic regulatory peptides in thyroid C cells of different mammals. Gen. Comp. Endocrinol. **187**, 6–14.

Vaughan, J., C. Donaldson, J. Bittencourt, M. H. Perrin, K. Lewis, S. Sutton, R. Chan, A. V. Turnbull, D. Lovejoy, C. Rivier, P. E. Sawchenko, and W. W. Vale, 1995: Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotrophin-releasing factor. Nature **378**, 287–292.

Yang, L. Z., P. Tovote, M. Rayner, J. Kockskamper, B. Pieske, and J. Spiess, 2010: Corticotropin-releasing factor receptors and urocortins, links between the brain and the heart. Eur. J. Pharmacol. **632**, 1–6.