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Urocortinergic system in the testes of normal and cryptorchid dogs

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ABSTRACT

Cryptorchidism is the most common disorder of the sexual development in dogs, occurring in 13% of the males. Unilateral cryptorchidism is more frequent than bilateral and the right testis seems to be more frequently affected. Urocortin (UCN) is a corticotrophin-releasing hormone (CRH)-related peptide which was observed to affect several functions in male genital organs. The aim of the present study was to investigate the expression of UCN, and its receptors CRHR1 and CRHR2 by immunohistochemistry, Western blot and real-time RT-PCR in the normal and cryptic testis of the dog. The results showed that UCN, CRHR2 and CRHR1 were expressed in normal and cryptic testes. UCN-immunoreactivity (IR) was distributed in germ cells of the normal and cryptic testis. In the normal testis, CRHR2-IR was found in germ and interstitial Leydig cells. In the cryptic testis CRHR2-IR was distributed in gonocytes and interstitial Leydig cells. CRHR1-IR was distributed in the vessel smooth musculature and peritubular myoid cells. UCN and CRHR2 mRNA expression levels were lower in the cryptic than in normal testes. These results suggest that UCN and its receptors might play a role in regulating the spermatogenesis and hormonal activity of interstitial Leydig cells of the dog testis.

1. Introduction

Cryptorchidism is of major importance among the pathologies that affect reproduction in dogs as well as other animal species. It is caused by the failure of one or both testes to descent toward the scrotum. The descent is necessary to allow for the normal process of spermatogenesis and, therefore, male fertility (Moon et al., 2014; Foresta et al., 1996). The term cryptorchid means hidden testis; a non-castrated male with no testes present in the scrotum is a bilateral cryptorchid, whereas a male with only one testis in the scrotum is a unilateral cryptorchid. Unilateral cryptorchidism occurs more commonly than the bilateral condition. The unilateral cryptorchidism affected the right testis. In the dog, as in man, maldescended testes may affect fertility and predispose to neoplasm (Romagnoli, 1991; Memon and Hibary, 2001). Retained testes are smaller; the diameter of the seminiferous tubules is reduced by up to 60% compared to those of scrotal testes (Kawakami et al., 1988). A unilaterally cryptorchid animal can produce viable sperm, whereas a bilateral cryptorchid male is usually sterile. Testes should be scrotal (4–5 °C cooler than body temperature) to produce normal sperm.

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). The effects of urocortins are mediated by CRH receptor 1 (CRHR1) and CRHR2 which belong to the G-protein-coupled receptor superfamily of brain-gut neuropeptides (Perrin et al., 1993; Vita et al., 1995). However, it has been proven that urocortins have multiple effects, which include the ability to activate cellular metabolic pathways, thereby influencing the functioning of the central nervous system, as well as the cardiovascular, gastrointestinal, reproductive and immune systems (Neufeld-Cohen et al., 2010; Zorrilla et al., 2003; Fekete and Zorrilla, 2007; Kageyama, 2013; De Bonis et al., 2012). In the male reproductive system, the presence of UCN was reported in the human prostate, suggesting its role in the autocrine/paracrine regulation of prostatic function (Arcuri et al., 2002). Moreover, expression of UCN and CRH receptors was found in the rat, mouse and human testis (Lee et al., 2011; Tezval et al., 2009; Tao et al., 2007) and in the epididymis of the rat and alpaca (De Luca et al., 2014; Liguori et al., 2015). UCN and CRH receptors are believed to play a role in the regulation of spermatogenesis, sperm motility and testosterone releasing.

Despite these evidences, there are no indications available on the expression of UCN in the dog testis. To our knowledge, no data are available regarding the presence and putative roles of UCNs in the testis of the normal and cryptorchid dog.

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The aim of the present study was to investigate the expression of UCN, CRHR1 and CRHR2 in the normal and cryptic testis to verify the existence of a regulatory system based on the UCN and CRHRs in the testis in the normal and cryptorchid dog. For this purpose, we performed Western blot and immunohistochemistry to study the presence and distribution of these proteins and real-time RT-PCR to evaluate mRNA expression levels in the normal and cryptic testis of the dog.

2. Materials and method

2.1. Animals and tissue collection

This study was performed using five adult normal male dogs and five male dogs affected by unilateral cryptorchidism coming from surgery unit of the Department of Veterinary Medicine and Animal Productions of the University of Naples "Federico II". All the dogs were medium sized and aged between 2 and 8 years. Testes were collected immediately after bilateral orchiectomy by surgical techniques. Tissue samples were divided in three groups: *normal testis* (testis from normal dogs), *contralateral testis* (scrotal testis from dog affected by unilateral cryptorchidism) and *cryptic testis* (retained testis from dog affected by unilateral cryptorchidism). For Western blot and RT-PCR analyses, fresh segments of testis were immediately frozen on dry ice and stored at −80 °C. For immunohistochemical studies, fresh segments of testis were immediately fixed.

2.2. Immunoprecipitation and Western blotting

Tissue samples were homogenized in a buffer (50 mM Tris–HCl pH 7.00; 150 mM NaCl; 2% Triton; 5 mM EDTA; 10 lg/ml leupeptin; 0.1 U/ml aprotinin; 1 mM PMSF) using an Ultra-Turrax homogenizer and centrifuged at 16 000 × *g* for 20 min at 4 °C. 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. Beads were sedimented by brief centrifugation and washed extensively with ice-cold homogenization buffer. Proteins, solubilized in boiling sodium dodecyl sulphate (SDS) sample buffer (2% SDS; 5% L-mercaptoethanol; 66 mM Tris pH 7.5; 10 mM EDTA), were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto immunoblot nitrocellulose membrane as described elsewhere (Squillacioti et al., 2011). After 1 h blocking with 5% BSA and 0.3% Tween 20 in Tris-buffer saline (TBST), the membrane was incubated for 2 h at room temperature with primary antisera. The following primary anti-sera were used: polyclonal rabbit anti-UCN (U4757, diluted 1:1000, Sigma, St. Louis, MO, USA); anti-CRHR1 (SAB4500465, diluted 1:1000; Sigma) and anti-CRHR2 (SAB4500466, diluted 1:1000; Sigma). The membrane was washed three times with TBST, incubated for 1 h with anti-rabbit 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 visualized by an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK) and the image was acquired with the Kodak Gel Logic 1500 imaging system (Celbio, Milan, Italy). Marker proteins (colored protein molecular weight markers; Prosieve, Lonza) were used to estimate the molecular weight of each band.

2.3. Immunohistochemistry

Fresh fragments of testis 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. Immunohistochemical staining was performed by means of EnVision system-horseradish antiperoxidase (HRP) (cod. K4002, Dako, Santa Barbara, CA). After dewaxing in xylene and rehydration, sections were washed in phosphate buffered saline (PBS) and then placed in target retrieval solution (Citric buffer pH 7.4) brought to boil using microwave. Sections were washed with PBS and treated with 3% H_2O_2 (20 min), washed with PBS pH 7.4 and incubated in a humid chamber for 24 h at 4 °C with primary antibodies. Primary anti-sera were the same described in detail in the precedent section and were directed against UCN, CRHR1 and CRHR2 (diluted 1:1000). After incubation, the sections were washed in PBS and incubated with EnVision for 30 min at RT. The sections were washed and the immunoreactive sites obtained were visualized by incubation for 5 min in a fresh solution of

3,3′-diaminobenzidine tetrahydrochloride (DAB) (Vector). 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,

2.4. RNA extraction, cDNA synthesis and real-time RT-PCR

Tissue samples were homogenized in ice-cold TRI-Reagent (Sigma) using an Ultra-Turrax homogenizer. After chloroform extraction and isopropyl alcohol precipitation, RNA was dissolved in RNAase-free DEPC water. Total RNA was measured with an Eppendorf Biophotometer (Eppendorf AG, Basel, Switzerland). For cDNA synthesis, 1 mg of total RNA was retro-transcribed with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA) using random hexamers as primers. For conventional and real-time PCR reactions, specific primers were designed from the published mRNA Genbank gene sequences For PCR reactions, specific primers for dog UCN, CRHR1 and CRHR2 were designed from the published gene sequences *Canis lupus familiaris* UCN, mRNA (Genbank accession number XM_848667), *Canis lupus familiaris* corticotropin releasing hormone receptor 1 (CRHR1), transcript variant X6, mRNA, (Genbank accession number XM_005624199) and *Canis lupus familiaris* corticotropin releasing hormone receptor 2 (CRHR2), transcript variant X6, mRNA (Genbank accession number XM_005628687) using the Primer Express software (PE Applied Biosystems). The sequences of the primers are listed in Table 1.

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). The PCR products of dog UCN, CRHR1and CRHR2 were purified using GFX PCR DNA and Gel Purification Kit (28-9034-70, GE Healthcare, Little Chalfont, Buckinghamshire, UK) and sequenced. To assess the expression profiles of these genes, quantitative RT-PCR was used. The real-time PCR reactions contained 1 μl cDNA (20 ng/well) and 24 μl of SYBR Green Master Mix (Applied Biosystems) containing specific primers. The PCR conditions were 50 °C for 2 min and 94 °C for 10 min, followed

Table 1
Primer sequences for real-time RT-PCR.

Primer	Sequence
Sense UCN	5′-TCCGCTGTCCATTGATCTCAC-3′
Antisense UCN	5′-GGGACGGGGTCGAGGTTATC-3′
Sense CRHR1	5′-TCTTTCTGCGGCTCAGGAGTA-3′
Antisense CRHR1	5′-ACCTGCACCAGCCCACATT-3′
Sense CRHR2	5′-GCGAAATGTCATGTGGTTCCT-3′
Antisense CRHR2	5′-GCGCTCAGTGGAGTACGTCAT-3′
Sense beta-actin	5′-CGGCATCGTCACCAACTG-3′
Antisense beta-actin	5′-CGTCACCGGAGTCCATCA-3′

by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. The beta-actin gene was also amplified in separate tubes under the same conditions to serve as an active endogenous reference to normalize quantification of the mRNA target. Real-time detection was performed on an ABIPRISM 7300 Sequence Detection System (Applied Biosystem), and data from the SYBR Green I PCR amplicons were assessed with ABI 7300 System SDS Software. The relative quantification method 2-deltadeltaCt ($2^{-\Delta\Delta Ct}$) was used for the normalization of gene expression as described more fully elsewhere (Squillacioti et al., 2011). For statistical analyses, the data were expressed as mean ± SD. Significant differences in the UCN, CRHR1 and CRHR2 mRNA levels between the calibrator sample (normal testis) vs. contralateral and cryptic testis were determined by one-way ANOVA followed by Tukey's HDS test for independent samples. The level of statistical significance was set at $p < 0.05$ for all experiments

3. Results

3.1. Immunoprecipitation and Western blotting

The results of the immunoprecipitation and Western blot analysis are shown in Fig. 1. Tissue extracts of the normal, contralateral and cryptic testis of the dog reacted with the anti-UCN, -CRHR1 and CRHR2 antibodies. Tissue extracts reacted with the anti-UCN antibody (Fig. 1A). The antibody recognized one major protein band of approximately 16 kDa from tissue extracts and one protein band of approximately 6.5 kDa from the synthetic peptide.

The homogenates also reacted with the anti-CRHR1 and anti-CRHR2 antibodies (Fig. 1B and C). The antibodies recognized one protein band measuring approximately 55 kDa.

3.2. Immunohistochemistry

In the normal testis, UCN-immunoreactivity (IR) was distributed in germ cells located on the basement membrane of the seminiferous tubule (type A and type B spermatogonia) (Fig. 2A) and in some pachitene spermatocytes and spermatocytes in meiosis. The distribution of UCN-IR in the contralateral testis was similar to normal testis (Fig. 2B). Seminiferous tubules of the cryptic testis were composed mostly by Sertoli cells. Spermatogonia and spermatocytes were relatively few. Gonocytes were numerous. These cells were large with abundant and strongly eosinophilic cytoplasm. UCN-IR was found in gonocytes and spermatocytes (Fig. 2C).

In the normal testis, CRHR1-IR was found in vascular musculature and in the peritubular myoid cells (Fig. 2D). In addition, CRHR1-IR was distributed in some spermatocytes in meiosis (Fig. 2D). The distribution of CRHR1-IR in the contralateral testis was similar to normal testis (Fig. 2E). In the cryptic testis, CRHR1-IR was observed only in the vascular musculature (Fig. 2F).

In the normal testis, CRHR2-IR was found in numerous pachitene spermatocytes, in some round spermatids and in Leydig cells (Fig. 2G). The distribution of CRHR2-IR in the contralateral testis was similar to normal testis (Fig. 2H). In the cryptic testis, CRHR2-IR was observed in pachitene spermatocytes and gonocytes (Fig. 2I). In addition, CRHR2-IR was found in Leydig cells (Fig. 2I).

3.3. Real-time RT-PCR

Real-time RT-PCR experiments confirmed that UCN, CRHR1 and CRHR2 mRNAs were expressed in normal, contralateral and cryptic testis of the dog (Fig. 3). The levels of UCN and CRHR2 mRNA expression were lower in cryptic testis than in normal and contralateral testis (Fig. 3). The levels of CRHR1 mRNA expression were the same in the all examined samples (Fig. 3).

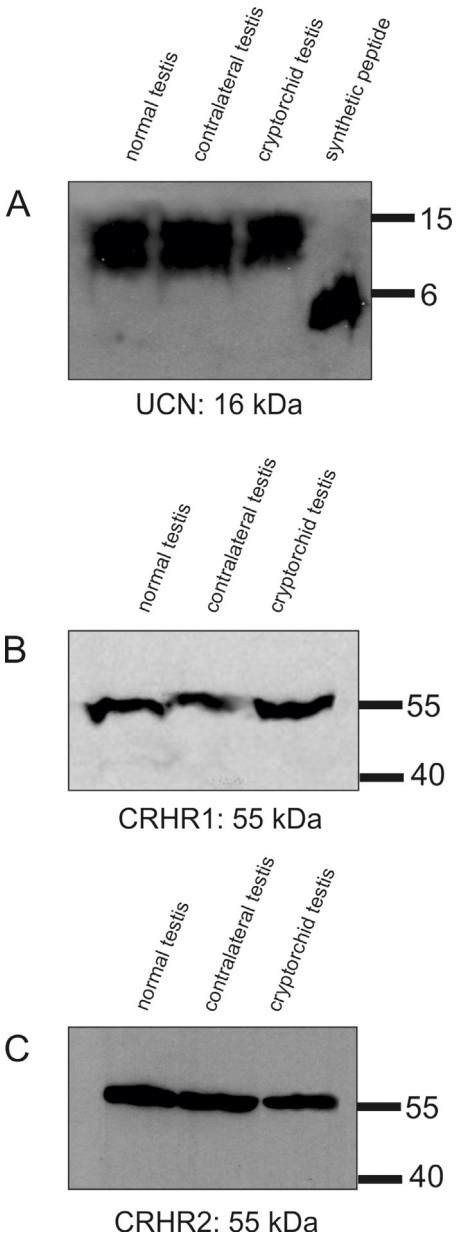

Fig. 1. Detection by Western blot analysis of UCN, CRHR1 and CRHR2 in the normal, contralateral and cryptic testis of the dog. (A) UCN (16 kDa) was detected in the normal, contralateral and cryptic testis. Synthetic UCN peptide (6 kDa) was also detected as a positive control. (B) CRHR1 (55 kDa) was detected in the normal, contralateral and cryptic testis. (C) CRHR2 (55 kDa) was detected in the normal, contralateral and cryptic testis.

4. Discussion

The results of the present study demonstrated that UCN, CRHR1 and CRHR2 are expressed in the normal, contralateral and cryptic testis of the dog. The protein band of approximately 16 kDa detected by immunoprecipitation and Western blot is consistent with the mammalian UCN precursor, which is a 122-aa protein (Vaughan et al., 1995; Donaldson et al., 1995). In addition, 55 kDa protein bands are consistent with mammalian CRH receptors (Perrin et al., 2006).

In this study, UCN-IR was found in the spermatogonia and spermatocytes of normal and in some gonocytes of the cryptic testis. UCN, except for some spermatocytes, usually was not colocalized with its receptors. Previously, the presence of UCN, CRHR1 and CRHR2 mRNAs and IRs were demonstrated in the mouse testis (Tao

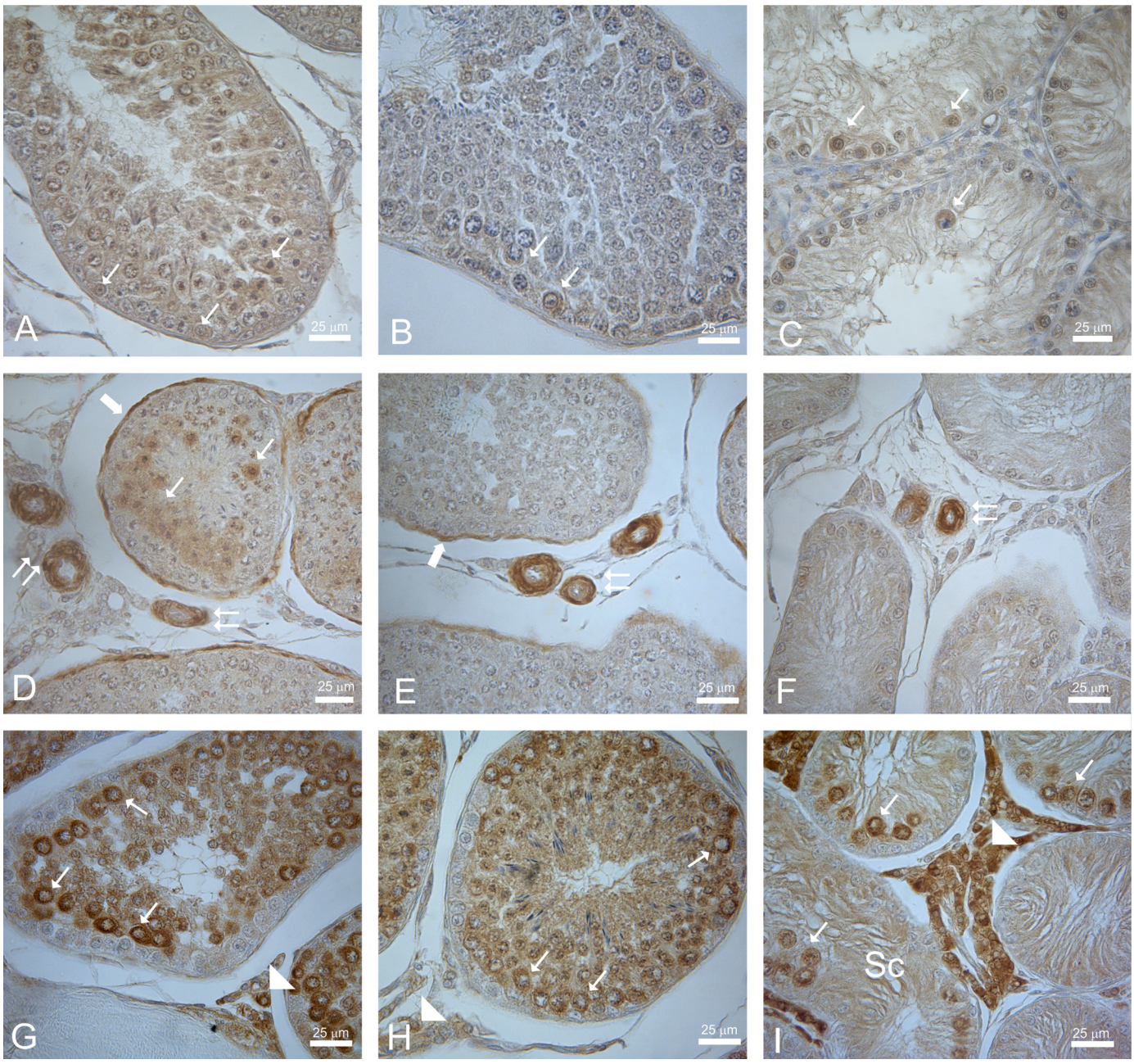

Fig. 2. Distribution of the immunoreactivity to UCN (A–C), CRHR1 (D–F) and CRHR2 (G–I) in the normal (A, D, G), contralateral (B, E, H) and cryptic (C, F, I) testis of the dog. In the normal testis UCN-IR was distributed in the germ cells (↑) and CRHR2-IR was found in the germ cells and interstitial Leydig cells (▲). In cryptic testis UCN- and CRHR2-IRs were found in gonocytes (↑) and in interstitial Leydig cells. CRHR1-IR was found in the vessel smooth musculature (⇑) and in the fibromuscular cells encircling testicular tubules (⬆) in the normal and cryptic testes. Sc Sertoli cells. Scale bar: 25 μm.

et al., 2007). UCN, CRHR1 and CRHR2 IRs were found to be distributed in the testicular germ cells, where UCN seemed to inhibit spermatozoa motility and acrosome reaction by inhibition of Ca^{2+} channels (Tao et al., 2007). In the human testis, UCN and CRHRs mRNAs and IRs were found to be separately located among germ, peritubular myoid and Leydig cells, and UCN was suggested to have receptor-independent effects on germ cell differentiation and division (Tezval et al., 2009). In the rat testis, the presence of UCN mRNA and IR appears to be restricted to Leydig cells (Lee et al., 2011; Rivier, 2008). Spermatogenesis is a complex biological process consisting of proliferation of spermatogonia, meiosis of spermatocytes and differentation of spermatids into spermtozoa. The spermatogonia are testicular staminal cells that are regulated for proliferation and differentiation during spermatogenesis. A balance between spermatogonial stem cells self-renewal and differentation is therefore essential to maintain normal spermatogenesis and fertility (for a review Garcia and Hofmann, 2015). In the testis, the regulation of the germ cell homeostasis is governed by endocrine and paracrine mechanism via soluble factors including hormones, growth factors, POMC (proopiomelanocortin) peptides, PModS ("Peritubular Modifies – Sertoli) and cytokines (review Skinner et al., 1991; review Chen and Liu, 2015). Urocortin has been shown to have antiproliferative and antiapoptotic effects (Carlson et al., 2001; Wang et al., 2008; Chatzaki et al., 2006). It can be hypotized that UCN plays a role in the germ cells differentiation by autocrine/paracrine mechanism. In the cryptic testis, UCN was localized in the gonocytes, germ cells that not begin the spermatogenesis. Cryptorchidism usually leads to clinical infertility due to severe decrease in sperm production. It

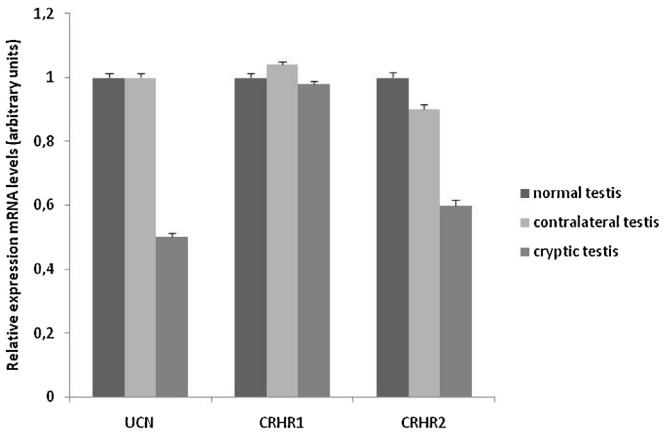

Fig. 3. UCN, CRHR1 and CRH-R2 mRNA expression in the normal, contralateral and cryptic testis of the dog by real-time RT-PCR. The statistical differences in UCN, CRHR1 and CRHR2 mRNA levels between the calibrator sample (normal testis) and the contralateral and cryptic testis were determined by one-way ANOVA followed by Tukey's HDS test. For UCN (*) $p < 0.001$ normal testis vs cryptorchyd testis and contralateral testis vs cryptorchid testis; (#) $p = 0.9$ normal testis vs contralateral testis. For CRHR1 (#) $p = 0.97$ contralateral testis vs cryptorchid, $p = 0.98$ contralateral testis vs normal testis; $p = 0.99$ normal testis vs cryptorchid testis. FOR CRHR2 (**) $p < 0.05$ normal testis vs cryptorchid testis and contralateral testis vs cryptorchid testis; (#) $p = 0.94$ contralateral testis vs normal testis.

has been demonstrated that germ cells apoptosis is an important regulating process in the normal and cryptic testis (Sinha Hikim et al., 2003; Vera et al., 2004; Absalan et al., 2010). In the mouse testis, germ cell apoptosis induced by experimental cryptorchidism is mediated by molecular pathways involving proapoptotic and antiapoptotic genes as Bcl-2, Bax, p53 and survivin (Absalan et al., 2010). UCN is a antimitotic and antiapoptotic factor by using different signaling pathways (cAMP and Ca^{2+}) (Carlson et al., 2001; Wang et al., 2008; Chatzaki et al., 2006). It can be hypotized that UCN plays a role in the germ cell apoptosis induced by cryptorchidism.

CRHR1-IR was observed in the vascular smooth musculature, in peritubular myoid cells and in some spermatocytes in meiosis of the normal testis. In the cryptic testis, CRHR1 was localized only in the smooth muscle of the blood vessels. CRHR1-IR was distributed in the vascular smooth musculature, thus suggesting an involvement of CRH-related peptides in the modulation of the dog testicular blood flow. The flow of the blood in the testis is very important for the transport of the steroid hormones. This hypothesis is supported 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. CRHR1-IR was found in the peritubular myoid cells suggesting that CRH-related peptides are involved in the regulation of the contraction of the seminiferous tubules and in the cell-cell interactions. Cell-cell interactions mediated by factors such as androgens, POMC peptides, and PModS are all primarily directed at the regulation of cellular differentiation. In fact, it was been found that, under androgen regulation, testicular peritubular cells produce a paracrine factor, PModS that modulates Sertoli cell functions that are essential for the process of spermatogenesis (Skinner et al., 1991; Roser, 2008). The presence of CRHR1 in the germ cells suggests that CRH-related peptides play a role in the regulation of apoptosis. Additionally, other CRHR1 ligands, such as CRH probably interact with this receptor to regulate this mechanism. CRH induced apoptosis through reducing the Bcl/Bax ratio via CRHR1 while UCN2 exerted the opposite effect via CRHR2 (Jin et al., 2011).

In this study CRHR2-IR was found in germ and interstitial cells of the normal and cryptic testis suggesting that CRH-related peptides play a role in the regulation of the spermatogenesis and steroidogenesis. The presence of CRHR2-IR in the germ cells indicated that UCN via CRHR2 could be involved in the regulation of cell proliferation and differentation. In addition, CRHR2 binds also UCN2 and we do not esclude that this peptide affect these cells also. UCN2 inhibits apoptosis through increasing the Bcl/Bax ratio via CRHR2 (Jin et al., 2011). UCN is an antimitotic and antiapoptotic factor by using different signaling pathways (cAMP and Ca^{2+}) (Carlson et al., 2001; Wang et al., 2008; Chatzaki et al., 2006). The presence of CRHR2 in the Leydig cells of the normal and cryptic testis suggests that UCN play a role in the regulation of steroidogenesis via CRHR2 by a paracrine mechanism. It has been demonstrated that the intratesticular injection of CRH-related peptides, and in particular UCN, significantly inhibited the testosterone response to human chorionic gonadotropin (hCG) (Rivier, 2008). In cryptorchid dogs, LH-induced testosterone secretion is lower in the interstitial cells of retained than scrotal testes (Pathirana et al., 2011). On the other hand, there is an increase of the plasma levels of oestrogens in dogs with experimentally induced cryptorchidism (Kawakami et al., 1999). Thus it may be suggested that the reduced testosterone level is a result, not only of decreased synthesis of this steroid, but also of increased conversion of androgens into oestrogens. It has been reported that the plasma estradiol-17beta (E2) levels in the spermatic vein of the abdominal cryptorchid testis of dogs with congenital cryptorchidism are higher than in normal dogs (Mattheeuws and Comhaire, 1989). In addition, an increase in E2 production in the artiphicially cryptorchid testis has been observed in the rat (Damber and Bergh, 1990). Androgen–estrogen balance is very important for reproductive functions. It may therefore be suggested that hormonal imbalance is one of reasons for degenerative changes in the cryptorchid testes. The functional role played by testicular UCN in cryptorchid testis characterized by blunted androgen levels therefore needs to be further investigated.

RT-PCR analysis revealed that UCN, CRHR1 and CRHR2 mRNAs were expressed in the normal and cryptic testis of the dog thus confirming immunohistochemical data. The levels of UCN and CRHR2 mRNA expression were higher in the normal than in the cryptic testis. It has been demonstrated that in the dog retained testes, there is an increased risk of neoplasms, such as Sertoli cell tumors and seminoma, which can exhibit more aggressive behavior than those in scrotal testes (Hayes et al., 1985; Quartuccio et al., 2012). In addition, spontaneous unilateral cryptorchidism increases activity of Sertoli cells which may be a predisposing factor for Sertoli cell cancer in dog cryptorchid testes (Moon et al., 2014). UCN has been demonstrated to inhibit tumor growth and angiogenesis in hepatocellular carcinoma via CRHR2 (Wang et al., 2008). It can be hypothesized that the decrease of UCN and CRHR2 mRNA levels in the dog cryptic testis is an inducing factor for the occurrence of testicular tumors and that UCN has a protective effect. This hypothesis is also supported by the evidences that UCN mRNA was significantly reduced in human endometrial carcinoma versus healthy controls, and UCN peptide was not found in neopalstic samples (Florio et al., 2006).

In conclusion, these results clearly demonstrated that UCN, CRHR1 and CRHR2 are expressed in the normal and cryptic testis of the dog and that a local regulatory system exists based on UCN and its receptors. In particular, UCN may play a role in the regulation of mitotic and apoptotic events occurring during spermatogenesis and in the regulation of the steroid hormones synthesis by an autocrine/paracrine mechanism. In addition, the decrease of UCN and CRHR2 in the cryptic testis suggests a role for UCN in preventing testicular tumors.

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