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Molecular cloning, sequencing, and distribution of feline GnRH receptor (GnRHR) and resequencing of canine GnRHR

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ABSTRACT

GnRH receptors play vital roles in mammalian reproduction via regulation of gonadotropin secretion, which is essential for gametogenesis and production of gonadal steroids. GnRH receptors for more than 20 mammalian species have been sequenced, including human, mouse, and dog. This study reports the molecular cloning and sequencing of GnRH receptor (GnRHR) cDNA from the pituitary gland of the domestic cat, an important species in biomedical research. Feline GnRHR cDNA is composed of 981 nucleotides and encodes a 327 amino acid protein. Unlike the majority of mammalian species sequenced so far, but similar to canine GnRHR, feline GnRHR protein lacks asparagine in position three of the extracellular domain of the protein. At the amino acid level, feline GnRHR exhibits 95.1% identity with canine, 93.8% with human, and 88.9% with mouse GnRHR. Comparative sequence analysis of GnRHRs for multiple mammalian species led to resequencing of canine GnRHR, which differed from that previously published by a single base change that translates to a different amino acid in position 193. This single base change was confirmed in dogs of multiple breeds. Reverse transcriptase PCR analysis of GnRHR messenger RNA in different tissues from four normal cats indicated the presence of amplicons of varying lengths, including full-length as well as shortened GnRHR amplicons, pointing to the existence of truncated GnRHR transcripts in the domestic cat. This study is the first insight into molecular composition and expression of feline GnRHR and promotes better understanding of receptor organization, and distribution in various tissues of this species.

1. Introduction

Neuroendocrine pathways affecting reproduction are complex with major players in the brain, pituitary gland, and gonads. Hormones secreted into the bloodstream affect the pathways by binding to receptor molecules expressed on specialized cells. Characterization of the hormone-receptor pairs participating in these pathways has led to a better understanding of reproduction in general and has been crucial for development of therapies that improve fertility or approaches that result in contraception. One of the major players in the neuroendocrine pathways affecting reproduction is GnRH1. GnRH1 is a small hypothalamic decapeptide that specifically binds to the GnRH type 1 receptor (referred from here on as GnRHR). The GnRHR is a member of the superfamily of G protein-coupled receptors with seven transmembrane domains [1]. The primary location of GnRHR is the pituitary gland where the receptors are restricted to gonadotrope cells [2,3]. Through GnRH binding, the receptor regulates the synthesis and secretion of pituitary gonadotropins that are essential for gametogenesis and synthesis of gonadal steroids. The GnRH peptide sequence is

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identical in the vast majority of mammalian species, except guinea pig [4]. Sequencing of the GnRHR in multiple mammals, on the other hand, determined the presence of some species-specific regions as well as extensive areas with close sequence similarities. Although GnRHR sequences for more than 20 mammalian species have been identified, no GnRHR sequence information is available pertaining to the domestic cat (*Felis catus*) or any other member of the Felidae family. This is rather surprising, because the domestic cat has been utilized for many years as a model to study reproductive processes for wild felids and humans, including regulation of ovarian follicle development [5], cryopreservation of male and female gametes [6], embryo transfer, and artificial insemination [7].

Outside of the pituitary, GnRHR expression (messenger RNA [mRNA] and/or protein) has been reported in reproductive and nonreproductive organs, including ovary, mammary gland, testis, prostate, hypothalamus, heart, urinary bladder, kidney, and liver. However, knowledge about the receptor molecular configuration and function in extrapituitary tissues remains fragmented and very limited. Based on immunolocalization of GnRHR protein in human ovary, Choi et al. [8] proposed a role of GnRHR in follicular development and corpus luteal function. Sengupta and Sridaran [9] evaluated GnRHR mRNA and protein expression in oviduct at time points important for the maintenance of pregnancy in rats. The results pointed to an autocrine/paracrine role of oviductal GnRH mediated by the receptor. To outline extrapituitary roles for GnRHR, several studies quantified GnRHR expression under various physiologic conditions. For example, Coit et al. [10] demonstrated that sterilization was associated with increased levels of GnRHR mRNA in urinary bladder of male and female dogs. This effect was most pronounced in female dogs with urinary incontinence, suggesting that these two conditions might be related. Expression of GnRHR in rat thymus after immunization against GnRH showed that GnRHR plays a pivotal role as an immunomodulator in thymus [11]. Albertson et al. [12] reported GnRHR within the mammalian cerebellum suggesting a connection between cerebellar ataxia and hypogonadotropic hypogonadism in patients with Gordon Homes and Boucher-Neuhauser syndromes.

The primary aims of this work have been to clone, sequence, and characterize GnRHR of the domestic cat and to study the distribution of GnRHR mRNA in various feline tissues. Comparative analysis of the feline GnRHR cDNA sequence to that of multiple mammalian species (including dog) highlighted a difference in a conserved region of the canine receptor. This observation led to resequencing of the canine GnRHR, an additional aim of this study. Resequencing of the receptor resulted in detection of a single base change compared with the published sequence of canine GnRHR cDNA [13] and confirmation of this change in different canine breeds.

2. Materials and methods

2.1. Animal samples

All tissue samples were collected at post mortem examinations from animals on several different research projects under protocols approved by Auburn University Institutional Animal Care and Use Committee. These protocols were not related to the present study. This was done in agreement with Auburn University animal care and use policies that permit post mortem tissue collection for unrelated research projects.

For molecular cloning and sequencing of the feline GnRHR cDNA, pituitary glands were obtained from three normal cats (a 6-month-old male and two females, 11 months and 3 years old). To sequence canine GnRHR cDNA, pituitary glands were collected from three normal female Beagle dogs (6 and 12 months and 8 years old). All tissues were flash frozen in liquid nitrogen and stored at −80 °C before use.

To study distribution of the feline GnRHR mRNA, multiple cat tissues (pituitary gland, heart, urinary bladder, liver, kidney, uterus, Fallopian tubes, ovary, testes, and prostate) were collected from four normal cats, two 6-month-old males and two females (1 and 7 years old). To ensure fair comparison between individual animals, tissues in all cats were collected from similar anatomic locations of each organ/tissue type. Samples were taken from the apex of the heart. Liver samples were taken from the caudal edge of the left lateral lobe. Urinary bladder samples were cranial sections of the bladder wall. For the kidney, axial slices were taken approximately 0.5 cm from the caudal pole of the left kidney and included both cortex and the medulla. Entire organs/tissues were collected for gonads, prostate, and Fallopian tubes. Uterus samples included portions of uterine horns close to the uterine body. Tissue samples were collected using a separate set of sterile tools for each tissue to avoid cross-contamination of RNA. To prevent RNA degradation, each tissue was placed in a sterile Petri dish containing RNAlater Solution (Ambion, Life Technologies Corp., Grand Island, NY, USA) and diced quickly. After that, three or four tissue fragments of approximately 100 mg each were transferred into 2-mL screw cap tubes with fresh RNAlater solution and kept refrigerated overnight to allow RNAlater to penetrate the tissues. Tubes with tissues were then stored at −20 °C. Processing and storage of tissues in RNAlater was performed according to the manufacturer's recommendations.

2.2. Sequencing of feline and canine GnRHR cDNA

For feline GnRHR sequencing, total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Germantown, MD, USA). The RNA concentration was determined by the absorbance at 260 nm using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). Using a SuperScript III First Strand Synthesis System kit (Invitrogen, Carlsbad, CA, USA), total RNA was reverse transcribed to first strand cDNA. The 5′ and 3′ ends of the feline GnRHR cDNA were amplified using FirstChoice RLM-RACE Kit (Ambion) and sequenced with the primers A and B, respectively. These and additional primers used in this study for feline and canine cDNA sequencing are shown in Table 1. All primers were synthesized by TIB MOLBIOL, LLC (Adelphia, NJ, USA). Based on the obtained sequences of the 5′ and 3′ ends, feline GnRHR-specific primers, C and D, were designed to amplify the entire feline GnRHR cDNA. The primers incorporated EcoR1 and Xba1 restriction sites that were later used for cloning of