Animal Reproduction Science 178 (2017) 31–39

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Expression and localization of guanine nucleotide-binding protein alpha S in the testis and epididymis of rams at different developmental stages

Zhen Li[a], Jieli Lu[a], Jia Chen[b], Quanhai Pang[a,*], Ruipeng Nan[a], Zhiwei Zhu[c]

[a] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, PR China
[b] Institute of Farm Product Storage and Fresh-keeping, Shanxi Academy of Agriculture Science, Taiyuan, PR China
[c] College of Life Science, Shanxi Agricultural University, Taigu, Shanxi, PR China

ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form 9 January 2017
Accepted 14 January 2017
Available online 15 January 2017

Keywords:
Guanine nucleotide-binding protein alpha S (*Gnαs*)
Ram
Testis
Epididymis

ABSTRACT

The guanine nucleotide-binding alpha S subunit (Gαs) is an important element of key signaling pathways, which is widely expressed in mammalian tissues; however, its role in the reproductive system is still unclear. In this study, we investigated the expression and localization of Gαs in the testes and epididymis of rams at different developmental stages using quantitative RT-PCR, immunohistochemistry, and western blotting. In 1-, 6-, and 12-month-old rams, the transcription of Gαs-encoding gene (*Gnαs*) was significantly upregulated in the corpus and cauda epididymis compared to the testes and caput epididymis ($P < 0.05$). At 12 months, the level of *Gnαs* mRNA was higher than that at 1 and 6 months for all tested tissues ($P < 0.05$). The Gαs protein was detected in the principal cells and interstitial epididymal cells, including Sertoli and Leydig cells, as well as in testicular cells, spermatogonial stem cells, and spermatocytes. Gαs expression was the highest in the cauda epididymis ($P < 0.05$), followed by the corpus epididymis, caput epididymis, and testes. The results indicate that in the reproductive organs of rams, Gαs is expressed in a tissue-specific and age-dependent manner. The high levels of Gαs observed in the epididymis suggest that Gαs may influence the composition of the epididymal lumen fluid and, consequently, the microenvironment for spermatozoa maturation. Thus, Gαs could play an important role in spermatogenesis and the development of the testes and epididymis in the reproductive system of rams.

1. Introduction

The guanine nucleotide-binding alpha S (Gαs) protein encoded by the *Gnas* locus is a widely expressed G protein subunit (Weinstein et al., 2007) playing a key role in many signaling pathways (Choi et al., 2009), as it independently transduces signals from a number of G protein-coupled receptors to important signaling molecules, including adenylate cyclase, phospholipase Cβ, and ion channels (Simon et al., 1991). Upon binding to its cognate receptors, Gαs activates adenylyl cyclase and induces the generation of cAMP, a secondary messenger, which in turn activates cAMP-dependent protein kinase A (PKA) (Simon et al., 1991). Through this pathway, Gαs is able to induce the transcription of approximately 4000 target genes (Simon et al., 1991; Zhang et al., 2005), including those regulating apoptosis. Although Gαs is expressed in

* Corresponding author at: College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Minxian Road No. 1, Taigu, Shanxi 030801, PR China.
E-mail address: pangquanhai@163.com (Q. Pang).

http://dx.doi.org/10.1016/j.anireprosci.2017.01.006
0378-4320/

Table 1
Oligonucleotide primers for the *Gnαs* genes.

Gene	GenBank accession No.	Primer sequences (5′-3′)	Product length (bp)
Gnαs (bovine, long)	NM_181021.3	GGGTGCGTGCCTGCTATGA GGCGGATGTTCTCGGTGTC	633
Gnαs (ovine, short)	GAAI01007748.1	CTATGTGCCCAGCGACCAG GATCCATTTGCGGCGTTC	139

most tissues, it demonstrates tissue specificity, and is primarily synthesized in the pituitary, thyroid, renal proximal tubules, gonads, and hypothalamus through maternal alleles (Weinstein et al., 2010). Gαs has been detected in the heart, liver, and spleen of humans (Li et al., 2000), cows (Khatib, 2004), (Weinstein et al., 2004; Tang et al., 2008; Zhang et al., 2012), and mice pigs (Peng et al., 2014), and is also expressed in the ovaries of humans (Li et al., 2000), cows (Li et al., 2000), rats (Weinstein et al., 2004), and landrace pigs (Oczkowicz et al., 2013). Rey et al. (2006) found that Gαs was expressed in human testicular Sertoli and Leydig cells, where it may regulate cell division and the expression of Anti-Müllerian hormone (AMH) and LH-stimulated steroid hormones through PKA activation. Gαs also participates in other regulatory pathways related to the biological activity of estrogens and androgens (Rey et al., 2006; Oczkowicz et al., 2013; Sang et al., 2014). These findings indicated that Gαs plays an important role in the process of gonadal development. Although a number of studies focusing on Gαs functional activity have been conducted in humans and animals, few of them were performed in traditional livestock such as sheep. In particular, there are no reports on Gαs expression and localization in the testes and epididymis of sheep at different stages of development. In this study, we used 1-, 6-, and 12-month-old rams obtained by crossing Poll Dorset rams with Mongolian sheep ewes to examine the Gαs expression profile in the testes and epididymis at different developmental stages to further understanding of the role of Gαs in the biological function of gonads.

2. Materials and methods

2.1. Test animals and tissues

In this study, we used 18 healthy crossbred rams obtained by crossing Poll Dorset rams with Mongolian ewes, provided by the Taifeng breeding park, Datong City, Shanxi Province, China. Three age groups were analyzed: 1-, 6-, and 12-month-old animals (n = 6 per group), with the average body weight of 3.0 ± 0.5 kg, 36.5 ± 0.6 kg, and 51.5 ± 1.2 kg, respectively. Testes together with the caput, corpus, and cauda epididymis were collected into cryopreservation tubes, quickly placed in liquid nitrogen, and preserved at −80 °C until RNA extraction (Shi et al., 2010). Another set of tissue samples (approximately 0.5 cm × 0.5 cm × 0.2 cm in size) were collected from the same sections of each ram and fixed in 4.0% formaldehyde for immunohistochemistry analysis. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the institutional ethics committee.

2.2. Total RNA extraction, cDNA synthesis, and conventional PCR

Total RNA was extracted and purified (TaKaRa Bio Co. Ltd., Dalian, China) from the testis and epididymis, and analyzed for concentration and purity using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). RibonucleicAcid (RNA) concentration was adjusted to approximately 1 μg/L, and the samples were either stored at −80 °C or used to obtain cDNA by reverse transcription performed using the PrimeScript® RT Reagent kit (TaKaRa Bio Co. Ltd., Dalian, China) according to the manufacturer's instruction. The reaction was conducted in a total volume of 10 μL containing 2.0 μL of 5 x Prime Script® Buffer, 1.0 μL of total RNA template, and 7.0 μL of RNase-free ddH_2O, at the following conditions: 37 °C for 15 min and 85 °C for 5 s. Samples were stored at −20 °C until analysis. *Gnαs*-specific primers were designed using the Primer Premier 5.0 software based on bovine and ovine *Gnαs* mRNA sequences (Table 1) and synthesized by Beijing BGI Life Tech Co., Ltd. (Beijing, China). PCR was conducted in a total volume of 15 μL containing 0.6 μL of forward and reverse primers (10 μmol), 0.8 μL of cDNA template, 5.5 μL of ddH_2O, 7.5 μL of 2 x Es Taq Master Mix (Real-Times Biotechnology Co., Ltd., Beijing, China). Cycling conditions were: 94 °C for 3 min; at least 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; and 72 °C for 5 min. Amplification products were detected by electrophoresis in 2% agarose gels.

2.3. Cloning, plasmid extraction, and sequencing

The recovered DNA fragments were ligated to the T3 Cloning Vector (TransGen Biotech., Beijing, China) at 25 °C for 10 min and then overnight (12–16 h) at 37 °C, according to the manufacturer's instructions. The ligation product was introduced into DH5α competent cells, which were spread on an ampicillin (AMP)/Luria-Bertani (LB) plate and cultured at 37 °C overnight (12–16 h). White colonies were selected and inoculated into LB broth for 12–16 h. Plasmids were purified using a plasmid purification kit (Sigma-Aldrich Co. LLC., Darmstadt, Germany) according to the manufacturer's protocol, and the concentration and purity of the plasmids were analyzed using the ND-1000 spectrophotometer. The constructs were sequenced by BGI Life Tech Co., Ltd.