TRANSCRIPTIONAL REGULATION OF THE PORCINE TYPE II GONADOTROPIN-RELEASING HORMONE (GnRH) RECEPTOR GENE

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Introduction

To date, 2 mammalian isoforms of GnRH have been identified, GnRH I and II. GnRH I, the original decapeptide, is known to play a critical role in the regulation of reproduction. Specifically, GnRH I binding to its receptor, GnRHR I, induces the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). A second decapeptide that closely resembles GnRH I was first isolated from the chicken and since has been found in many species, from bony fish to humans.

Table 1. COMPARISION OF GnRH ISOFORMS.

GnRH I	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
GnRH II	pGlu-His-Trp-Ser-**His**-Gly-**Trp**-**Tyr**-Pro-Gly-NH_2

* Amino acids in bold are different from the classic mammalian decapeptide, GnRH I.

While the GnRH II receptor (GnRHR II) genes have been isolated in musk shrews and old world monkeys, the complete receptor is not present in rats and mice and is non-functional in sheep, cattle and chimpanzees (1, 2). Though the amino acid sequence of GnRHR II has approximately 40% identity to GnRHR I, GnRHR II is unique as cDNA sequence in the pig predicts 5- and 7-TM isoforms (1, 2). In the human, GnRHR II mRNA has been isolated; however, a full-length GnRHR II protein may not exist due to a premature stop codon and a frame shift mutation (1). Instead, it is predicted that the human GnRHR II is a unique 5- or 6TM G-protein coupled receptor (GPCR) utilizing a non-AUG start codon to overcome the frame shift mutation and a seleno-cysteine incorporated at the UGA codon instead of a stop codon (1). Studies by Kauffman et al. (3, 4) in musk shrews suggested that this receptor is involved in female mating behavior and energy balance. Additionally, 2345 bp of the marmoset monkey GnRHR II promoter has been isolated (5). Promoter deletions showed the region between -766/-665 bp enhanced promoter activity.

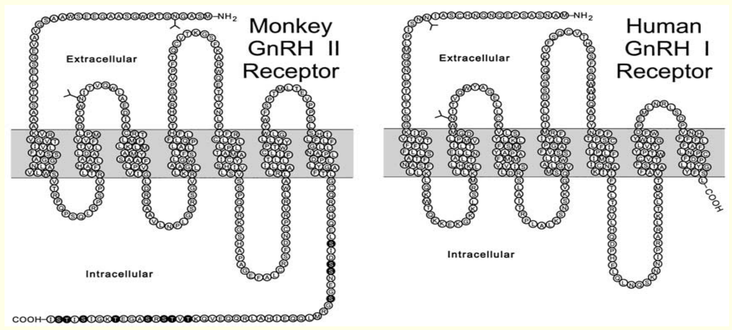

Figure 2. Structural diagram of the 7-transmembrane G-protein coupled receptors specific to GnRH I and II. While the amino acid sequences are 50% conserved between these receptors, the GnRHR II contains an intracellular C-terminal tail involved in receptor internalization that is absent in the GnRHR I. From Neill (2002).

We have isolated 3029 bp of 5' flanking region for the porcine GnRHR II gene. Transient transfections using vectors containing 3029 bp of 5' flanking region in a luciferase reporter vector indicated the promoter is active in both reproductive and non-reproductive cell lines. Similarly, analysis of reproductive and non-reproductive tissues using conventional PCR revealed the presence of GnRHR II mRNA in porcine tissues including: lung, skeletal muscle, anterior pituitary, hypothalamus, epididymis and testis.

Objectives

The aim of this study was to elucidate the transcription factors and corresponding binding sites contributing to basal activity of the porcine GnRHR II gene promoter in swine testis (ST) cells.

Materials and Methods

- **Vectors were constructed containing the full-length promoter for the porcine GnRHR II gene fused to the cDNA encoding luciferase. Additionally, block replacement constructs were prepared in the context of the full-length promoter by replacing the element of interest with a restriction enzyme site.**
- **Transient transfections were performed in swine testes (ST) cells using a liposome-mediated protocol (Fugene6, Roche Molecular). The reporter vector (0.9 µg) was cotransfected with a control vector, RSV beta-galactosidase (0.1 µg).**
- **Three transfections were completed in triplicate using DNA from three different plasmid preparations.**
- **Luciferase (Promega) and beta-galactosidase (Tropix) assays were performed.**
- **Luciferase values were divided by beta-galactosidase activity to normalize for transfection efficiency and means, expressed as fold changes over promoterless control, were compared using least significant differences.**
- **EMSAs were performed using 32P-labeled oligonucleotides spanning -707/-488 and crude nuclear extracts obtained from ST cells.**

Results

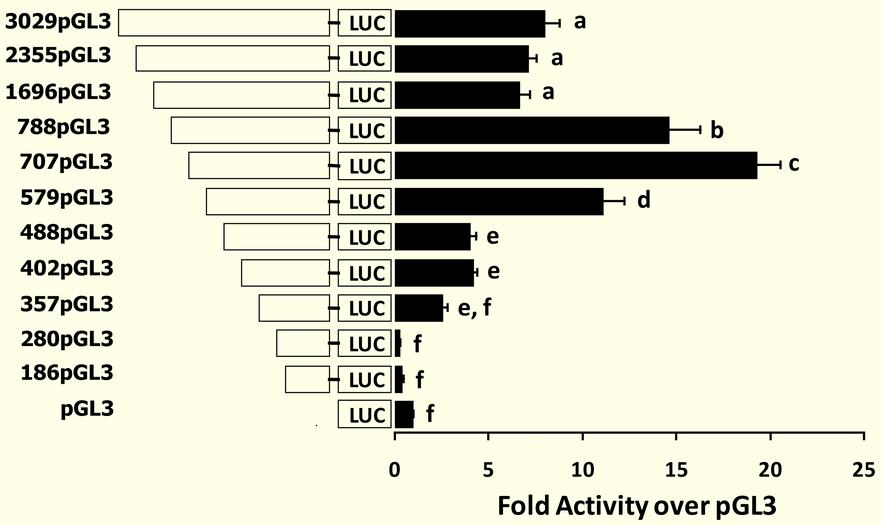

Figure 3. Critical 5' flanking regions conferring basal activity of the porcine GnRHR II gene promoter ST cells. Transient transfections were performed using luciferase (LUC) vectors containing 5' deletions of the 3029 bp of GnRHR II gene promoter. Bars with unique letters indicate differences (P<0.05).

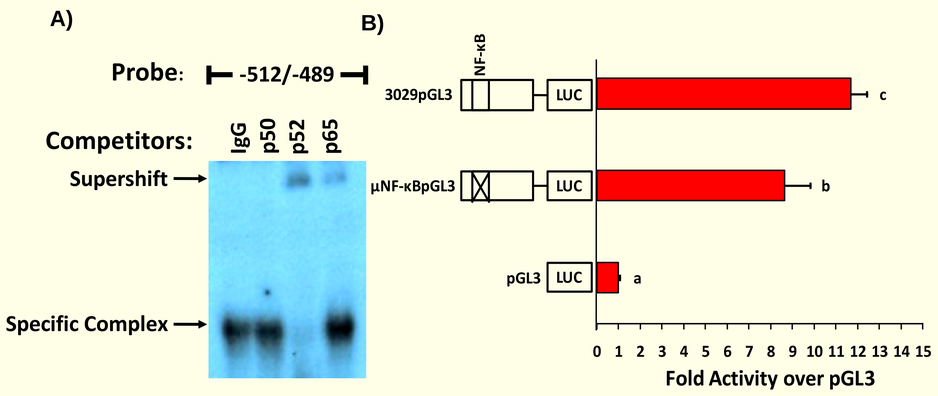

Figure 4. Contribution of a p52/p65 NF-κB heterodimer to basal activity of the porcine GnRHR II gene promoter. A) The p50 and p52 subunits of NF-κB comprise the specific binding complex. EMSAs performed using ST nuclear extracts show addition of antibodies directed against p65 and p65 subunits of NF-κB results in a shift of the DNA-protein complex. B) Transient transfections were performed using luciferase (LUC) vectors containing the 3029 bp of GnRHR II gene promoter (3029 pGL3), block replacement mutation of NF-κB site within 3029pGL3 (µNF-κBpGL3) or promoterless control (pGL3). Bars with unique letters indicate differences ($P < 0.05$).

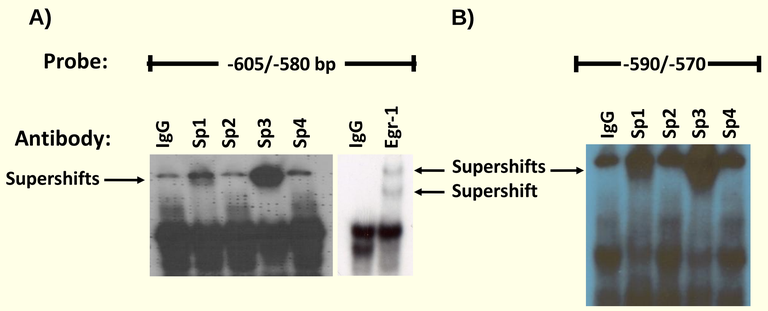

Figure 5. An Sp1/3 binding site and a Egr-1/Sp1/3 binding site located at -581/-574 and -596/-5586 bp of the porcine GnRHR II gene promoter, respectively. A) EMSAs performed using ST nuclear extracts show addition of antibodies directed against Egr-1, Sp1 and Sp3 results in supershifts of the specific binding complexes. B) EMSAs utilizing antibodies directed against Sp1 and 3 antibodies, but not 2 and 4, results in supershifts of the specific DNA-protein complex.

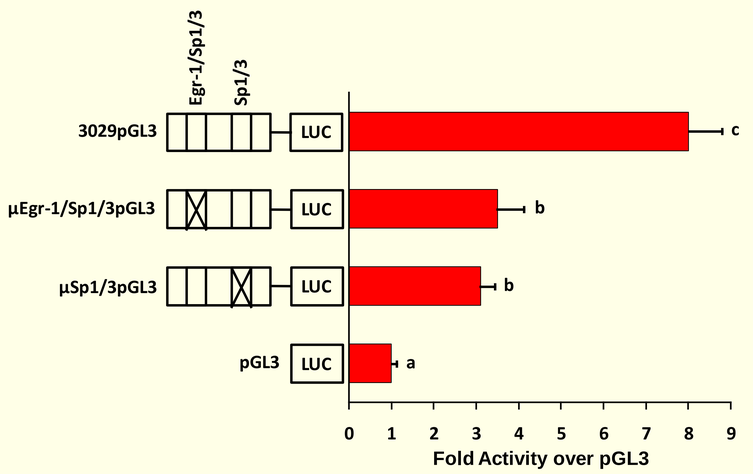

Figure 6. Specific contribution of Sp1/3 and Egr1/Sp1/3 binding sites to basal expression of the porcine GnRHR II gene in ST cells. Transient transfections were performed using luciferase (LUC) vectors containing the 3029 bp of GnRHR II gene promoter (3029 pGL3), block replacement mutation of Sp1/3 site within 3029pGL3 (µSp1/3pGL3), block replacement mutation of Egr1/Sp1/3 site within 3029pGL3 (µEgr1/Sp1/3pGL3) or promoterless control (pGL3). Bars with unique letters indicate differences ($P < 0.05$).

Conclusions

Activity of the porcine GnRHR II promoter in ST cells is largely conferred by NF-κB (-498/-492), Sp1/3 (-581/-574) and overlapping Egr-1/Sp1/3 (-596/-586) elements.

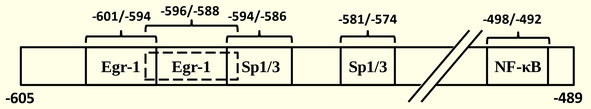

Figure 7. Working model for ST-specific activity of the porcine GnRHR II gene promoter.

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CTCCCGGGCTTGGCTCTGAGCACTGAAGCGGACCCAGGCGTCCATGACAGCGGCAGCCCC 60 bp
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CTCCCGGGCCTGGCTCTGAGCGCTGAAGCGGACCCAGGCGTCCATGACAGCGGCGGCCCC 60 bp

GGCTCGGCGGCCCCGGCTCCCGCCCCACTTCTCGCCCCTAGTCACGCCT 108 bp - Marmoset
||||| || |||||  | |||||||  ||||| |||||||||||||||||
GGCTCCGCAGCCCCACCCCCGCCC-ACTTCCCGCCCCTAGTCACGCCT 107 bp - Pig
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Figure 6. Alignment of partial 5' flanking regions for the marmoset (-766/-658 bp) and swine (-670/-562 bp) GnRHR II genes indicates 90% identity. Boxes surround conserved putative elements for Egr-1 and the Sp family of transcription factors, sequentially, within the marmoset GnRHR II promoter corresponding to functional binding sites located in the porcine GnRHR II promoter.

References:

1. **Neill et al., 2004. TRENDS in Endocrinology and Metabolism. 15:383-392**
2. **Millar 2003., TRENDS in Endocrinology and Metabolism. 14:35-43**
3. **Kauffman et al,. 2006. Endocrinology. 147:5069-5077.**
4. **Kauffman et al., 2005. Journal of Neuroendocrinology. 17:489-497.**
5. **Faurholm 2007. J Mol Endocrinol. 39(2):91-104.**
6. **Neill 2002. Endocrinology. 143(3):737-43.**