RELAXIN-3 AND RELAXIN/INSULIN-LIKE FAMILY PEPTIDE RECEPTOR 3 IN RAINBOW TROUT: SITES OF GENE EXPRESSION AND CHANGES IN MESSENGER RNA LEVELS DURING SPERMATOGENESIS IN TESTES.

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Background:

Relaxin is thought primarily to be a hormone that regulates pregnancy and reproduction in mammals. Recent studies have demonstrated the presence of six members of the relaxin family: Relaxin-1/2, -3, and insulin-like peptides-3, -4, -5 and -6. Relaxin-3 has recently been identified as a novel member of the insulin/relaxin family from human genomic databases [1]. In teleosts, genomic database searches revealed the existence of multiple relaxin-3-like genes [2, 3]. Relaxin-3 genes have been identified in the brain of eel and zebrafish [4, 5, 6], and were recently reported in the ovary of zebrafish and coho salmon [7, 8], suggesting that relaxin-3 like genes may play a role in oogenesis. However, little attention has been paid to the functions of relaxin-3 for male reproductive systems in teleosts. In order to explore the possibility of testicular functions of relaxin-3, we isolated cDNAs encoding relaxin-3 and relaxin/insulin-like family peptide receptor 3 from rainbow trout (*Oncorhynchus mykiss*). Sites of gene expression and seasonal changes in testicular relaxin-3 transcripts were investigated.

Methods:

Complimentary DNAs encoding rainbow trout relaxin-3 (rtRLN3) and relaxin/insulin-like family peptide receptor 3 (rtRXFP3) were identified from the brain. Sites of rtRLN3 and rtRXFP3 gene expression were examined by RT-PCR and *in situ* hybridization. Changes in levels of rtRLN3 and rtRXFP3 mRNA in testes were determined by quantitative real-time PCR throughout the reproductive cycle of male rainbow trout.

Results: Rainbow trout RLN3 transcripts were abundant in brain and found at lower levels in gonads. *In situ* hybridization analysis showed that the rtRLN3 transcripts were localized in the middle-posterior region of the brain, though the rtRLN3 transcripts were undetectable in the testes. The quantitative real-time PCR analysis showed that the levels of rtRLN3 transcripts were low during early spermatogenesis (January to February) and significantly increased 20-40 fold ($p<0.001$) during mid-spermatogenesis (March to May) just prior to significant increases in plasma 11-ketotestosterone [9]. The transcript levels then decreased in June prior to spawning ($p=0.0002$) and were maintained at low levels through the post-spawning phase (November). Rainbow trout RXFP3 transcripts were abundant in brain and immature gonads. The levels of rtRXFP3 transcripts were significantly elevated during early spermatogenesis in January ($p=0.0007$) and the post-spawning phase in November ($p=0.0049$). However, transcript levels were maintained at low levels through the spermatogenesis phase (February to October).

Conclusion:

Identification of the sites of rtRLN3 and rtRXFP3 gene expression provides a foundation for exploring the function of relaxin-3 in teleosts. Although the brain showed the highest transcript levels of rtRLN3 and rtRXFP3, the functions of relaxin-3 in brain still remain unclear. The seasonal changes in rtRLN3 transcripts in testes demonstrated that rtRLN3 gene expression was elevated prior to the significant increase of plasma 11-ketotestosterone [9]. Thus, the relaxin system may play a role in regulation of sex steroid synthesis during the early phase of gametogenesis [7]. However, there was no apparent correlation between the pattern of rtRLN3 and rtRXFP3 gene expression. Further experiments will be necessary to determine the precise functions of the relaxin system in male teleosts.

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