NOVEL NEUROPEPTIDES INVOLVED IN THE CONTROL OF PUBERTY AND REPRODUCTION IN FISH

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Introduction:

The findings that inactivation of kisspeptin signaling, because of mutations in the kisspeptin receptor, is associated with hypogonadotropic hypogonadism and absent or delayed puberty in man [1] stimulated the many subsequent studies that together have led to a new view of the neural control of GnRH release [2]. With this in mind, the recent observations [3] that mutations of the genes encoding neurokinin B (NKB) (*TAC3*) and its receptor (*TAC3R; NKBR*) were also associated with hypogonadotropic hypogonadism, is therefore of considerable interest. In order to study the physiological function(s) and evolutionary conservation of NKB, we cloned *tac3* and *tac3 receptor* cDNAs from several fish species, including zebrafish.

Methods:

Zebrafish tac3a, tac3b, tac3Ra and tac3Rb were cloned by synteny, according to Biran *et al.* [4]. A phylogenetic tree of all the currently known vertebrate neurokinin genes was generated with the neighbor-joining method. Real-time PCR and receptor transactivation assays were performed as previously described [4].

Results and Discussion:

A comparison of the zebrafish protein coding sequences of *tac3* cDNAs with those of the human and mouse TAC3 proteins showed identities of 23% and 24%, respectively. The phylogenetic tree showed that the vertebrate neurokinin genes fall into two distinct lineage groups. One lineage includes the mammalian and rodent TAC3, and all the piscine Tac3 that were cloned in the present study. The second lineage includes the mammalian and fish *tac1*. Nonetheless, high identity was found between different fish species, in the region encoding the NKB; all shared the common C-terminal sequence.

Many genes encoding the tachykinins have been found to encode a precursor that produces more than one tachykinin. However, in mammals TAC3 is unusual in that it encodes only a single tachykinin – the NKB. Interestingly, we have found that in zebrafish, exon 3 of both tac3s encodes a well conserved additional tachykinin. Furthermore, *in silico* analysis revealed that this new tachykinin exists in all other fish species whose genomes are known, and it was termed NKF. NKF also possesses the common C-terminal sequence FVGLM, known as the tachykinin signature.

zftac3a-expressing neurons were localized in specific brain nuclei that are known to be implicated in reproduction, whereas *zftac3b*-expressing neurons were more dispersed throughout the brain. Zebrafish *tac3a* – but not *tac3b* – mRNA levels gradually increased during the first few weeks of life, and peaked in fish with ovaries containing mature oocytes or with testes containing mature spermatozoa. Estrogen treatment of mature fish causes increase in *tac3a*, *kiss2* and *kiss1rb* expression in males, with no significant change in females.

Tac3Ra and tac3Rb transduce their activity via both PKC/Ca^{2+} and PKA/cAMP pathways. Both tac3 receptor types were very sensitive to amidation of their cognate ligands.

Conclusion:

These results indicate that the NKB/NKBR system may participate in puberty initiation in fish. Moreover, this novel system may be involved, in parallel with the kisspeptin system, in neuroendocrine regulation of GnRH secretion.

References:

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