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Structure and Expression of the gene encoding Piscine Vasoactive Intestinal Peptide (VIP) Precursor

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Introduction

VIP belongs to the glucagon-growth hormone-releasing factor secretion superfamily, and consists of 28 amino acids that is derived from a precursor protein of 170 amino acids by proteolytic cleavage. The sequence has been remarkably well conserved during the evolution from protochordates to mammals, suggesting an important biological function. VIP has been localized to neurons in the central and peripheral nervous systems where It functions as a neurotransmitter. Apart from the classical role of neurotransmitter, VIP also acts as a growth factor and therefore shows cytokine-like activities. Recently, VIP is widely considered to have a physiological regulatory influence on a side spectrum of body functions, being involved in the pathogenesis of several disorders, and offers hope for the improved prevention or treatment of certain diseases in human. Here, we report that the first piscine VIP precursor gene isolated from olive flounder, *Paralichthys olivaceus*.

Materials and Methods

Flounder VIP cDNA was isolated from the suppression subtracted cDNA library of PBL stimulated by LPS. The genomic structure of the VIP precursor genes was determined by sequencing PCR products obtained from cDNA primers and genomic DNA. DNA sequencing was performed using the BigDye Terminator Cycle Sequence Kit (Applied Biosystems) on an ABI3100 automated sequencer (Applied Biosystems).

Results and Summary

The entire coding region of VIP precursor spans 1,087-bp yielding a 148 amino acid primary translation product including a typical leader sequence of 21 residues. The 3′-UTR contains three RNA instability motif (ATTTA) that is found in typical of genes coding for inflammatory mediators. We present the full sequence of the VIP precursor gene for the teleost olive flounder (*Paralichthys olivaceus*), which consists of 6 exons and 5 introns. Using RT-PCR, flounder VIP transcript was detected in brain, intestine, stomach, spleen and eye of healthy flounder. We also examined the relationship VIP and inflammatory cytokines including tumor necrosis factor-α, interleukin-1β, and interleukin 6 though RT-PCR.

		Proteolytic cleavage site	
	РНІ	VIP	
Human	81 HADGVFTSDFSKLLGOLSAKKYLESLM	GKRVS-NISEDPVPVKRHSDAVFTDNYTRLRKQHAVKKYLNSILNGKRSSEGESPDFPEELEK	169
Mouse	B2 HADGVFTSDYSRLLGQISAKKYLESLI	GKRISSS1SEDPVPIKPHSDAVFTDNYTRLRKQMAVKKYLNSILNGKRSSEGDSADFLEELEK	171
Rat	81 HADGVFTSDYSRLLGOISAKKYLESLI	GKRISSSISEDPVPVKRHSDAVFTDNYTRLRKQHAVKKYLNSILNGKRSSEGDSPDFLEELEK	170
chimpenzee	HADGVFTSDFSKLLGOLSAKKYLESLM	GKRVSSNISEDPVPVKRHSDAVFTDNYTRLRKQMAVKKYLNSILNGKRRWGGSCQMKIIDESD	170
chicken	89 HADGITTSVYSHLLAKLAVKRYLHSLI	RKRVSSQDS-PVKRHSDAVFTDNYSRFRKQHAVKKYLNSVLTGKRSQEELNPAKLRGEAE	174
Xenopus	B7 HADGLETSGYSKLLGOLSARRYLESLI	GKRVNNNVVEDQVPVKRHSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEDIDPASLRENAD	176
Flounder	36 HADGLETSGYSKLLGOLSARRYLESLI	GKRVSDELMEEPVKRHSDAIFTDNYSRFRKOMAVKKYLNSVLTGKRSLEDPGTSDAEESRD	123
. 10 111111	27 amino acids	28 amino acids	

Figure 1. A comparison of amino acid sequences of VIP

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