Vasoactive Intestinal Peptide (VIP) Protects Nile Tilapia (Oreochromis niloticus) against Streptococcus agalatiae Infection

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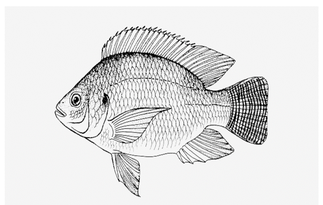
Introduction

Vasoactive intestinal peptide (VIP), a short peptide containing 28 amino acids be-longing to the secretin-glucagon family, is initially isolated from the gastrointestinal tract as a potent vasodilator peptide. The structure of VIP was similar to trypsin, pituitary adenylate cyclase activated polypeptide (PACAP), and glucagon, and particularly the homology between PACAP and VIP was more than 68%. VIP was initially identified in normal nervous tissue and neurons, and was subsequently recognized as a neurotrans-mitter widely distributed in various tissues. The wide distribution of VIP determines its involvement in a range of biological activities, such as gut motility, hormonal regula-tion, circadian rhythms, immune responses, and carcinogenesis



Oreochromis niloticus

VIP can rapidly react to the most toxic and intense stimulus, which also plays a pivotal role in physiology and pathology. To date, many studies have identified VIP and GPCRs in mammals and indicated that it plays a crucial part in neuro regulation, response to foreign stimuli, and maintenance of autoimmune balance . Moreover, VIP and receptors were also identified in some fish and their roles in reproduction were recorded . However, knowledge about the precise immunomodulation roles and mechanisms of VIP and receptors in bony fish is still lacking.

Nile tilapia (Oreochromis niloticus), a fish that is commercially significant in over 100 nations and regions, is a species that is very important for the world's aquaculture and widely farmed for international trade [19,20]. However, the outbreak of Streptococcus agala-tiae has resulted in vast losses in the tilapia industry over the past decade and severely in-fluenced the development of tilapia farming in China and around the world [20,21]. Therefore, this study aims to uncover the functions of VIP on the responses of Nile tilapia against S. agalatiae.In this study, VIP and VIPR1 gene from Nile tilapia were identified and characterized. Moreover, the expression profiles and immunological roles of VIP under S. agalatiae infection were assessed. These data will be the first to shed light on the roles of the secretin-glucagon family in fish innate immunity.

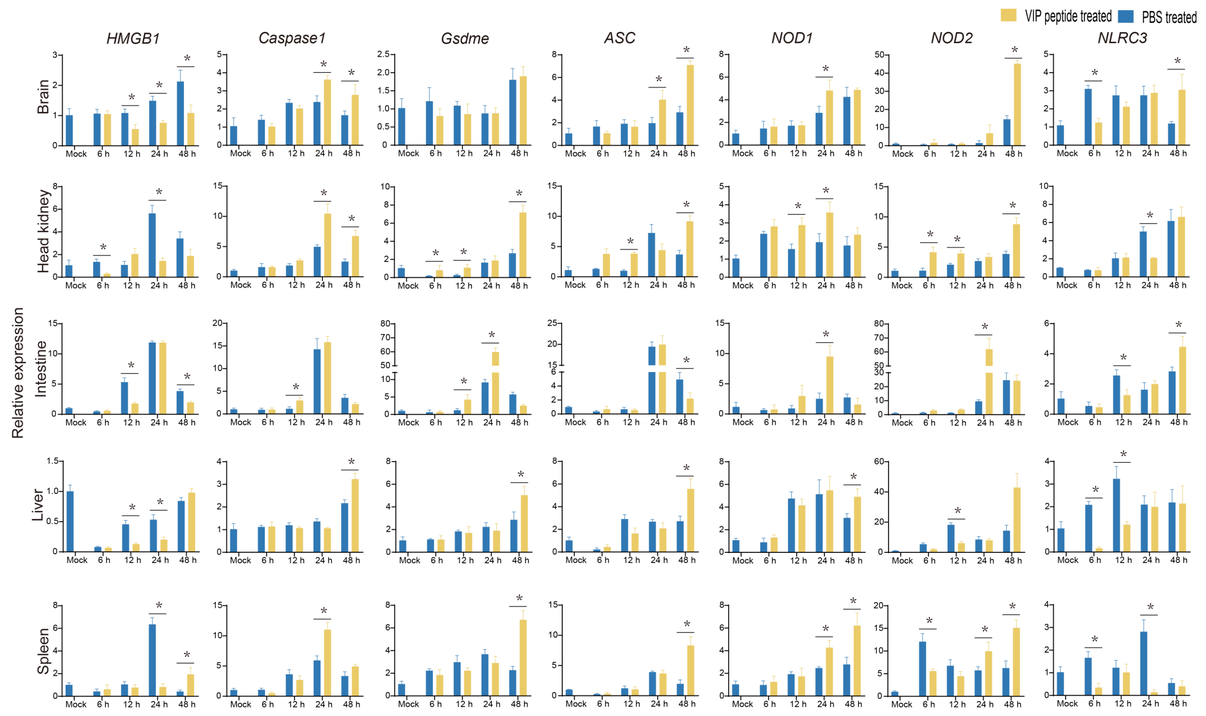


Figure. 6 The relative expression of pyroptosis-related factors (*HMGB1*, *Caspase1*, *Gsdme*, *ASC*, *NOD1*, *NOD2*, and *NLRC3*) at various points after *S. agalactiae* infection via qRT-PCR. Every value was presented as the mean and standard deviation; n = 3. The different letters were applied to reveal the significant difference ($p < 0.05$).

Results

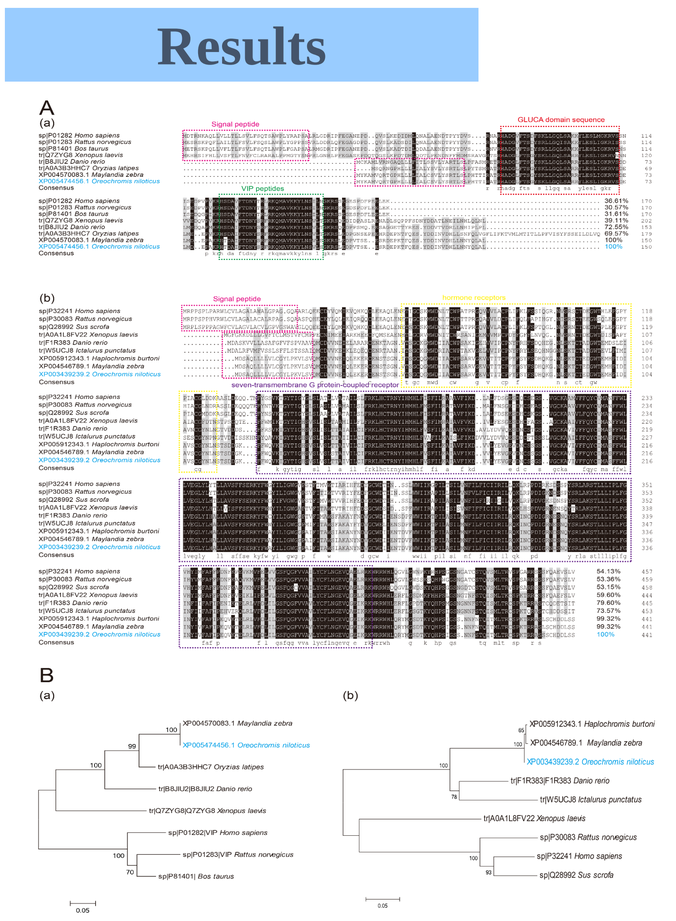


Figure 1. **(A)** The multiple alignments sequence of VIP (a) and VIPR1 (b) from several species. The signal peptide domain was marked in the pink dotted box. The GLUCA domain sequence and VIP peptides are marked in red and green dotted boxes, and the hormone receptors and seven transmembrane G protein-coupled receptors are marked in yellow and purple dotted boxes. **(B)** The phylogenetic trees of VIP and VIPR1 were created using the MEGA 6.0 software. On-VIP (a) and On-VIPR1 (b) are marked in blue.

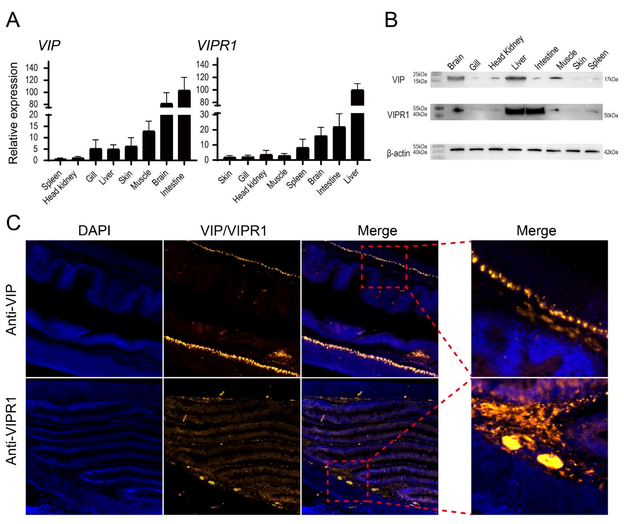


Figure 2. **(A)** By using qRT-PCR, the transcription level of On-VIP and On-VIPR1 in various ogans of unchallenged and healthy tilapia was identified. Every value is presented as a mean standard deviation; n = 3. The transcriptional levels of *On-VIP* and *On-VIPR1* in the spleen skin were set as 1, respectively. (B) By using Western blot, the protein level of On-VIP and VIPR1 in various organs of unchallenged and healthy tilapia were assessed. (C) The location of On-VIP and On-VIPR1 in the intestine of unchallenged and healthy tilapia

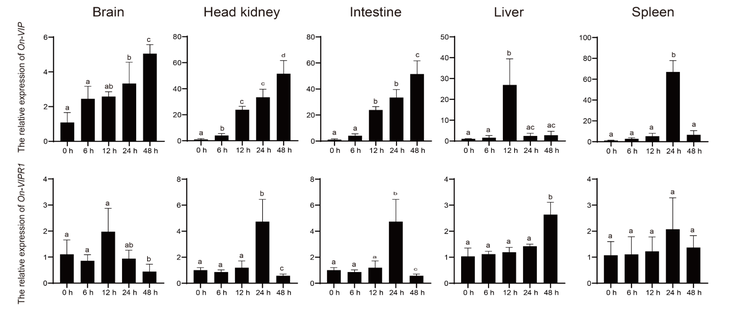


Figure 3. The transcriptional levels of *On-VIP* and *On-VIPR1* in the liver, head kidney, brain, spleen, and intestine of tilapia infected with S. agalactiae at various time points *via* qRT-PCR. The transcriptional levels of *On-VIP* and *On-VIPR1* at 0 h were set as 1. Every value was presented as the mean and standard deviation; n = 3. The different letters were applied to reveal the significant difference ($p < 0.05$).

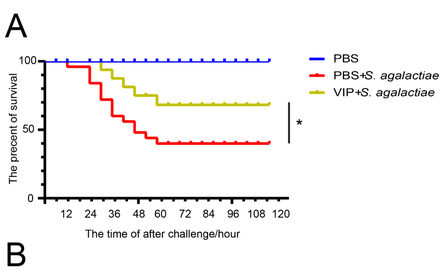


Figure. 4 **(A)** After S. agalactiae infection, the survival rates of Nile tilapia. The PBS treated Nile tilapia for control, deaths were counted every 6 hours for a total of 120 hours, n=100 for each group. Each fish in the PBS group, PBS + S. agalactiae group, and On-VIP + S. agalactiae groups were injected with 100 μL PBS, S. agalactiae, mixture of S. agalactiae and On-VIP peptide, respectively. Asterisks indicate a significant difference ($p < 0.05$). **(B)** At 24 h after S. agalactiae infection, the bacterial loads of the liver and spleen were detected. Every value was presented as the mean and standard deviation; n=6; ns was used to indicate no significant difference ($p > 0.05$)

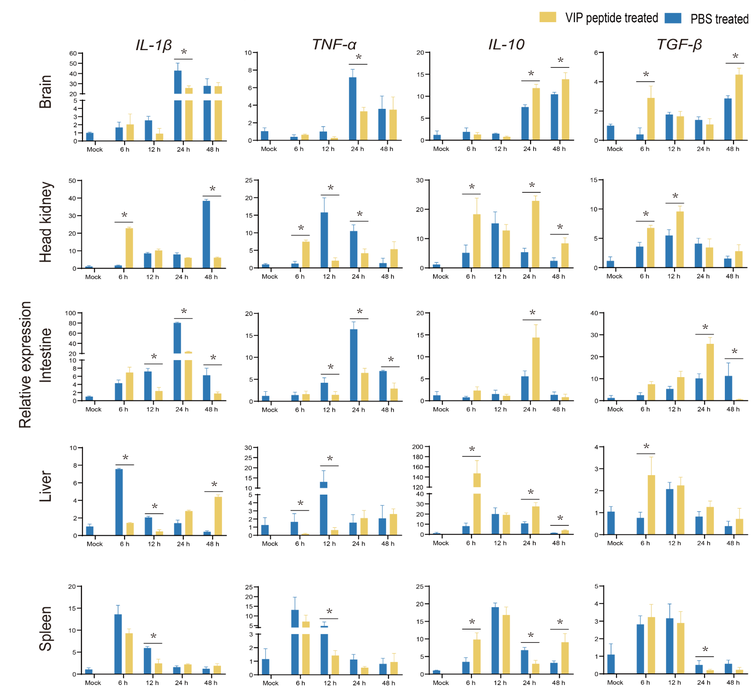


Figure. 5 The expression patterns of inflammatory-related factors (*IL-1β*, *TNF-α*, *IL-10*, and *TGF-β*) at various points after *S. agalactiae* infection via qRT-PCR. Every value was present-ed as the mean and standard deviation; n = 3. The different letters were applied to reveal the significant difference ($p < 0.05$).

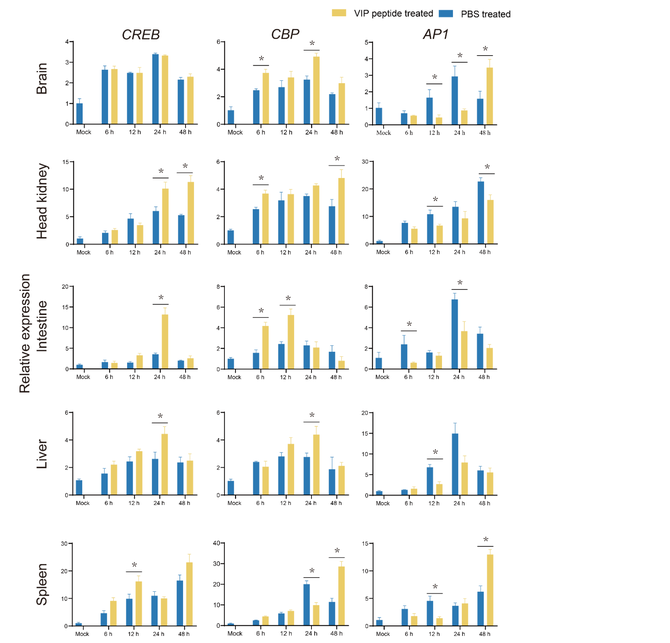


Figure. 7 The expression profiles of cAMP-PKA pathway-related factors (*CREB*, *CBP*, and *AP1*) at various points after *S. agalactiae* infection via qRT-PCR. Every value was presented as the mean and standard deviation; n = 3. The different letters were applied to reveal the significant difference ($p < 0.05$).

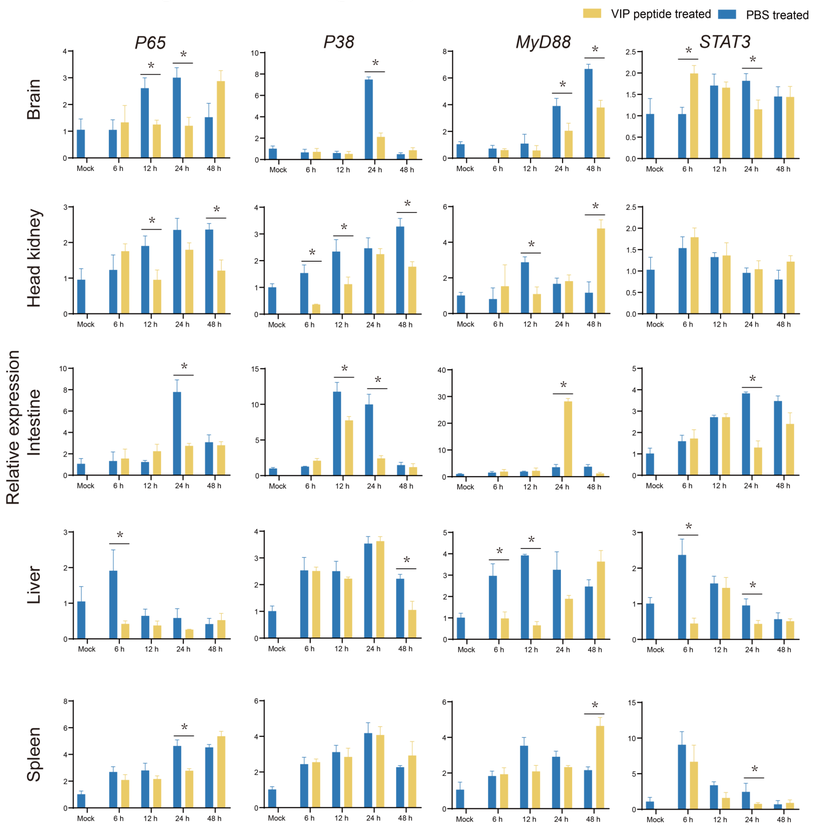


Figure. 8 The expression profiles of immune-related pathways (*P65*, *P38*, *MYD88*, and *STAT3*) at various points after *S. agalactiae* infection *via* qRT-PCR. Every value was presented as the mean and standard deviation; n = 3. The different letters were applied to reveal the significant difference ($p < 0.05$).

Conclusion

(1) On-VIP and On-VIPR1 contain a 450 bp and a 1326 bp open reading frame encoding deduced protein of 149 and 441 amino acids, respectively.
(2) The transcript of both On-VIP and On-VIPR1 were highly expressed in the intestine and sharply induced by Streptococcus agalactiae.
(3) The positive signals of On-VIP and On-VIPR1 were detected in the longitudinal muscle layer and mucosal epithelium of intestine, respectively.
(4) In vivo experiments indicated several immune functions of On-VIP, including reduction of *P65*, *P38*, *MyD88*, *STAT3*, and *AP1*, up-regulation of *CREB* and *CBP*, suppression of inflammation, and promotion of apoptosis and pyroptosis.

Acknowledgments

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