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Additive effects of orexin B and vasoactive intestinal polypeptide on LL-37-mediated antimicrobial activities

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ABSTRACT

The present study examined the bactericidal effects of orexin B (ORXB) and vasoactive intestinal peptide (VIP) alone or combined with cationic antimicrobial peptides, such as LL-37, on *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Staphylococcus aureus*. The bactericidal effect of ORXB or VIP alone was detected in low NaCl concentration, but attenuated in physiological NaCl concentration (150 mM). However, such attenuated bactericidal activities of ORXB and VIP in 150 mM NaCl were regained by adding LL-37. Therefore, our results indicate that VIP and ORXB appear to mediate bactericidal effects in concert with LL-37 in the physiological context of mucosal tissue.

1. Introduction

In general, neurons produce both a conventional chemical neurotransmitter and one or more neuropeptides. Neuropeptides function as neurotransmitters in the brain and the peripheral nervous system. However, they also play roles in regulating immune function and neurogenic inflammatory responses through vasodilatation, plasma extravasation, and recruitment of immunocompetent cells (Toriya et al., 1997; Jonsdottir, 2000). Furthermore, recent studies revealed that some neuropeptides released from the neuroendocrine system, such as Neurokinin-1 (NK-1) and neuropeptide Y (NPY), have antimicrobial properties (Brogden et al., 2005). Vasoactive intestinal polypeptide (VIP) is widely expressed in the central nervous system, as well as peripheral tissues, including the lung, stomach, skin, and oral cavity, where it has been shown to have a multitude of biological functions (Dickinson and Fleetwood-Walker, 1999; Awawdeh et al., 2002). VIP was recently reported to have direct antimicrobial activities against both Gram-positive and Gram-negative bacteria (El Karim et al., 2008). It is, therefore, plausible that the nervous system may deploy some antibacterial function in the form of released neuropeptides, such as VIP, whose delivery to innervated peripheral sites is considered to protect the nervous system from infection by microorganisms.

Orexins are novel hypothalamic neuropeptides that have been implicated in the regulation of feeding, arousal, and energy homeostasis (Kirchgessner, 2002; Ehrström et al., 2005). They are cleaved from a common precursor molecule, preopro-orexin, forming orexin A and orexin B (de Lecea et al., 1998; Sakurai et al., 1998). Since neurons and endocrine cells in the gut were found to display orexin-like immunoreactivity, it is implicated that orexins modulate the electrical properties and synaptic inputs of secretomotor neurons in the intestinal system and stimulate colonic motility (Kirchgessner and Liu, 1999). Besides the roles of neuronal activity mediated by orexins, it is also reported that orexin B (ORXB) modulates the function of peritoneal macrophages through activation of calcium-dependent potassium channels and induces enhancement of phagocytosis in mouse peritoneal macrophages (Ichinose et al., 1998; Ichinose and Watanabe, 2004). Interestingly, the chemical properties of ORXB, i.e., amino acid composition (28 amino acids), net charge, and isoelectric point, are very similar to those of VIP (Table 1). Since antimicrobial action of a peptide can be predicted by its chemical properties, especially amphipathic nature and cationic charge (Brogden et al.,

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Table 1
Characteristics of VIP, ORXB and LL-37.

Name	Sequence	AA[a]	Charge[b]	IP[c]
Vasoactive intestinal peptide (VIP)	HSDAVFTDNYTRLRKQMAVKKYLNSILN	28	3.1	10.2
Orexin B (ORXB)	RSGPPGLQGRLQRLLQASGNHAAGILTM	28	3.1	12.1
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	37	6.0	11.1

[a] Number of amino acids.
[b] Net charge in pH 7.0.
[c] Isoelectric point.

2005), it is plausible that ORXB may function as an anti-infective molecule. However, it has not been reported whether ORXB has antimicrobial properties.

Cationic antimicrobial peptides are evolutionarily conserved small proteins which play a critical role in the host innate immune defense system against microorganisms. Specifically, LL-37, the sole human cathelicidin, is widely expressed in a variety of bodily fluids and tissues, including key immune cell types, such as monocytes, neutrophils and lymphocytes, as well as epithelial cells on the mucosal surface (Durr et al., 2006; Hosokawa et al., 2006; Mookherjee et al., 2007). LL-37 facilitates a broad spectrum of antimicrobial activities against Gram-negative and Gram-positive bacteria, fungi and viruses. In addition, LL-37 has been reported to have synergistic or additive effects with other antibacterial agents (Chen et al., 2005). Especially, some large antimicrobial proteins, such as lysozyme and lactoferrin, appear to function in concert with LL-37 to kill *E. coli* (Singh et al., 2000). However, it is unclear if intestinally produced neuropeptides, namely, ORXB and VIP, can exert additive effects on LL-37-mediated antimicrobial function.

In the present study, we hypothesized that ORXB and VIP not only exert antimicrobial activity by themselves, but they also work in concert with other mucosal antimicrobial peptides, such as LL-37, to protect against infection in the context of intestinal tissue. To prove this hypothesis, the present study examined the antimicrobial activities of ORXB and VIP alone and combined with LL-37, HBD-1, and HNP-1 against Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus mutans*.

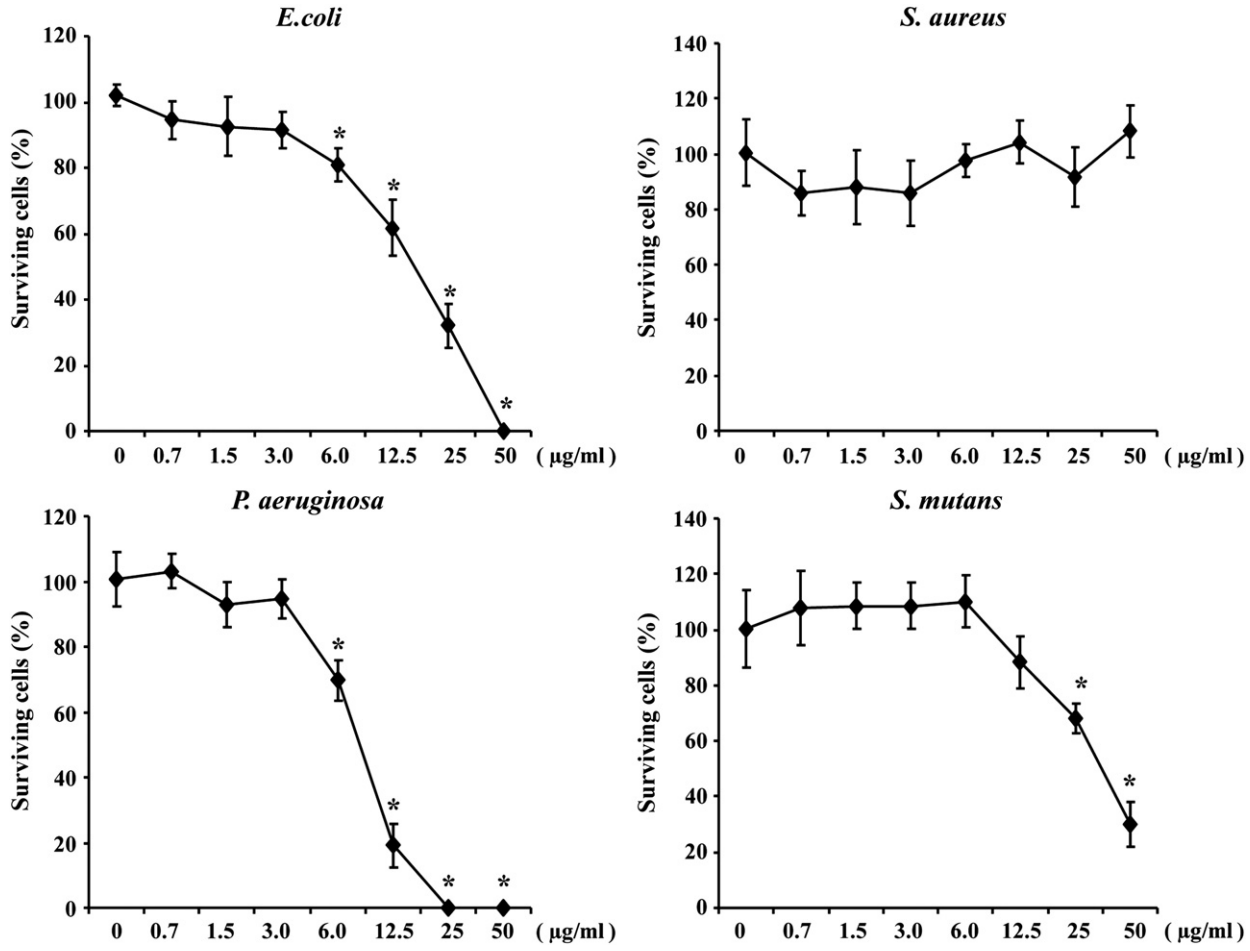

Fig. 1. Bactericidal activities of VIP. Bacterial cells were incubated with VIP for 2 h at 37 °C in 10 mM NaPi (pH 6.8). Then serial dilutions were plated on each agar, and colony counts were obtained after 24 h of incubation at 37 °C. Bacterial survival is expressed as a percentage (number of cells that survived in the presence of peptides compared to the number of cells that survived without a peptide). Data are shown as the mean ± standard deviation of three independent experiments. *Significantly different from non-treated bacterial cells (Student's *t*-test: $P<0.05$).