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Could Substance P be an antiviral mediator and potential therapeutic target in the nose?

Marianna Kulka, PhD,^{1,2}

¹ Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta T6G 2E1, Canada

² National Institute for Nanotechnology, National Research Council Canada, Edmonton, Alberta T6G 2M9, Canada

* Co-corresponding authors

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Corresponding author:

Marianna Kulka, PhD

National Institute for Nanotechnology, University of Alberta, 11421 Saskatchewan Dr, Edmonton, Alberta, CANADA T6G 2M9

Tel: 780-641-1687

Fax: 780-641-1601

E-mail: marianna.kulka@nrc-cnrc.gc.ca

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INTRODUCTION

Since its discovery by Von Euler and Gaddum in 1931, we have learned a considerable amount about substance P (SP) and its role in various human pathologies¹. Yet, much of the knowledge gained in animal studies has proven unreliable in human clinical trials. Originally described as a proteinaceous preparation that could induce hypotension and muscle contraction, it was later determined to be an undecapeptide, expressed by a number of cell types, including immune cells and neurons, and present in almost all body fluids, including blood, cerebrospinal fluid, and breast milk².

In the late 80s and early 90s, evidence that SP was an important participant in inflammation came from observations that inflamed tissues possessed increased amounts of SP and blockade of SP signaling, mainly using antagonists to its principal receptor neurokinin1 (NK1R), had some beneficial effects in inflammatory disease models³. Subsequent studies then showed that SP activated the production of proinflammatory mediators such as cytokines, chemokines, arachidonic acid metabolites and oxygen radicals, further supporting the premise that SP was a proinflammatory neuropeptide that facilitated communication between the nervous and immune systems. Thus, many animal models have shown that SP can have significant pro-inflammatory roles in certain contexts. Although various NK1R antagonists have proven very effective at ameliorating inflammation in various animal models, they have had little success in clinical trials and have proven ineffective in treating bronchoconstriction associated with asthma⁴. NK1R antagonists such as aprepitant have been successfully used to treat emesis associated with cancer chemotherapy, and there is growing evidence that they may be useful as an adjunct therapy for HIV patients, but their effectiveness as anti-inflammatories remains controversial⁵. This has prompted some to speculate that SP may also have unknown functions in human airways that may be unrelated to inflammation. At the

same time, viral infection increases SP production in a number of contexts⁶, and in the recent study in this issue by Larsson et al. titled “Substance P represents a novel first-line defense mechanism in the nose,” the authors suggest that SP may also influence innate immune responses, possibly via the toll-like receptor (TLR) system⁷.

TLRs have rightfully garnered a considerable amount of attention in recent years as the pathogen recognition receptors mediating several facets of a complex innate immune response. Relatively little information exists on the relationship between TLRs and SP, particularly in the airways and thus Larsson et al. explore the relationship between TLRs and SP in nasal epithelial cells and sensory neurons with the aim of determining whether SP could locally alter TLR expression and whether virus-like molecules could potentially alter SP production in nasal cells. They begin by examining the expression of TLRs in nasal epithelial cells in response to SP using a combination of immunocytochemistry and flow cytometry. The authors show that SP upregulated the expression of specific TLRs (namely, TLR1, TLR3, TLR4, TLR7 and TLR9) in human nasal epithelial cells and that this was at least partially mediated by activation of the NK1R. An examination of TLR expression in the nasal mucosa of healthy patients showed that TLR3, TLR4 and TLR9 were ubiquitously expressed throughout these tissues, confirming this group’s earlier observations that these TLRs were expressed in nasal epithelial cells⁸. In contrast to their earlier studies, the authors now examined the cellular contributors to the expression of these TLRs and found that TLR7 was limited to the nasal epithelium and nerve fibers. In cadaveric human trigeminal ganglia, they further discovered that TLR7 expression co-localized with SP expression on innervating nerve fibers. When human trigeminal ganglionic neurons (TGN), murine nasal epithelial cells (MNECs) and human nasal epithelial cells (HNECs) were stimulated with a TLR7 agonist R-837, they produced small but significant amounts of SP, further implying a link between these

two molecules. Perhaps not surprisingly, TGN produced far greater (about 500 fold) amounts of SP when compared to epithelial cells on a cell-by-cell basis. However, two observations were notable. First, SP production and release by all three cell types was very rapid – 15 min. This rapid release of intracellularly stored SP may occur *hie modus connaturalis* in neurons, but this would be unusual in epithelial cells which are not normally known to be mediator storehouses. Essentially, HNEC and MNEC produce and secrete SP very rapidly in response to the TLR7 ligand. As the authors point out, there are greater numbers of epithelial cells in the nasal mucosa compared to neurons, and epithelial cells could be a significant source of SP following TLR7 activation.

Our group has previously shown that SP upregulated TLR expression in human mast cells⁹, and work by other groups has shown that activation of TLR on nasal epithelial cells can impact innate immune responses¹⁰. In the study by Larsson et al., SP upregulated expression of TLR1, TLR3, TLR4, TLR7 and TLR9. With the exception of TLR1 and TLR4, these TLRs mainly respond to virally derived molecules. Therefore, SP could potentially act in an autocrine manner and upregulate TLRs on nearby epithelial cells. This rapid increase in expression of TLRs seems to be due to a change in localization of intracellular TLR4 to the cell surface as shown by confocal microscopy. TLR3, 7 and 9, on the other hand, reside both in the endoplasmic reticulum (ER) and in the Golgi complex in resting cells and traffics from these stores to the endosomes upon stimulation. The possible signaling pathways involved in such processes remain speculative, but there is some evidence that other stimuli are capable of inducing rapid intracellular shuttling of TLRs from intracellular stores to the surface. If this was to occur in HNECs, it would rapidly prime these cells to become increasingly sensitive to viral pathogen molecules.

These data provide a small glimpse of alterations of TLR expression following SP treatment and we still know very little of the mechanisms and regulatory networks involved. However, this study essentially makes two important observations. First, that HNEC express NK1R and respond to SP via NK1R by upregulating TLRs. Second, that HNEC and MNEC can very rapidly produce significant amounts of SP. With these two observations, the authors establish a cyclic relationship between neurons and epithelial cells in response to viral molecules, and by extension possibly viral infection. The hypothesis that nasal mucosal cells communicate with one another during a viral infection by producing SP is an attractive one because SP is rapidly produced by neurons (and also by nasal epithelial cells as observed in the present study) and relatively short-lived with a short half-life in tissues, making it a perfect candidate for rapid, localized signals. It is important to note, however, that these studies were performed using TLR agonists, not virus or virally-derived molecules. To adequately determine whether SP is protective during a viral infection, further work using a live respiratory virus needs to be performed, while also testing NK1R and TLR antagonists. The use of virally-induced asthma exacerbation models may also provide some important insights into the potential contribution of this communication network to virus-induced asthma.

Since SP is known to play an important role in airway inflammation associated with allergic disease, it is nevertheless tempting to speculate that this cyclic relationship specifically in the nasal mucosa is somehow involved in allergic rhinitis following a virally-associated exacerbation. Since this study obtained tissues from healthy human donors and mice, we have little information of how this relationship would respond in an “allergic inflammatory” situation. Perhaps increased SP levels would make allergic patients hyperreactive to subsequent viral infections via engagement of the TLRs, as the authors suggest. By extension,

a re-examination of NK1R antagonists in human airway disease in the context of viral infection may reveal a new role for these drugs in the clinic.

The increased expression of SP in the nasal mucosa may be a normal, potent and rapid part of the innate immune response to viral infection in the nose that may facilitate the clearance of viral particles. The nasal mucosa is a highly vascularized tissue that contains a large amount of mast cells and SP-producing sensory nerve fibers. Nasal bronchial epithelial cells express high amounts of NK1R on their apical surface, allowing them to respond rapidly to SP-mediated signals. Amplified production of SP in the nasal mucosa initiates increased plasma protein extravasation and this causes increased fluid and plasma protein release into the extracellular space, probably via activation of mast cells and their rapid release of histamine. SP can also stimulate the large number of mucous glands in the nasal mucosa and potentiate the cholinergic contraction of the smooth muscle fibers present around the acini and tubes in these glands, resulting in rapid mucus discharge. All of these physiological changes could result in some level of viral particle clearance from the nasal cavity, initiation of antimicrobial protein production from activated epithelium and promotion of tissue repair. In essence this may be an important part of the protective host response to viral infection in the nose.

Larsson et al. examined expression of TLR in response to SP treatment of nasal epithelial cells and further studies will need to determine whether this translates into increased sensitivity to virus particles and viruses. Although this process seems to require NK1R, other receptors for SP may be involved in different pathological settings. We have recently shown that SP can also activate the G protein-coupled receptor MrgprX2, and this receptor may also be involved in this process and concurrent blockade of both NK1R and MrgprX2 would

completely eliminate this response. The mechanism whereby NK1R shuttles intracellular TLRs to the cell surface and endosomes could contribute important information to the area of TLR research, particularly as it relates to autophagy and endosomal packaging of TLRs.

Lastly, it remains to be determined whether SP can similarly increase TLR expression on neurons, thereby completing the communication loop described in this study. This cross-talk between the nervous system and immune system would provide novel insights into the ever-expanding field of neuroplasticity, suggesting that viral infections may alter the host's production of neuropeptides and therefore alter the host's neuronal responses throughout their lifetime. This is very intriguing because it implies that, much like the immune system, the nervous system can also "learn and adapt" throughout a person's lifetime of exposure to infection.

Although this study requires a great deal of additional study, it does open some intriguing possibilities for therapies in the context of clinical disease. For example, further examination of SP effects on innate immune responses may reveal potential new uses of existing and FDA-approved neuropeptide modifiers, i.e. NK1R antagonists, as treatments for virus-associated disease. Conversely, SP and its signaling network may be attractive new targets for vaccine development, possibly using SP analogues as adjuvants for vaccines administered through the nasal mucosa. All of these statements are speculative, but offer innovative new approaches that may ultimately treat patients more effectively.

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