Int J Clin Exp Med 2018;11(12):12946-12955
www.ijcem.com /ISSN:1940-5901/IJCEM0072488

Review Article

Substance P and its role in viral infection

Wenya Xu[1*], Tongsong Wang[2*], Hongwei Zhou[1,3]

[1]Department of Ophthalmology, Lianshui County People's Hospital, Huai'an, China; [2]Department of Pharmacy and Biomedical Sciences, De Duve Institute, Université Catholique de Louvain, Brussels, Belgium; [3]School of Medicine, Southeast University, Nanjing, China. [*]Equal contributors.

Received September 6, 2017; Accepted September 10, 2018; Epub December 15, 2018; Published December 30, 2018

Abstract: Substance P (SP) is an 11-amino acid neuropeptide of the tachykinin family that preferentially activates the neurokinin-1 receptor (NK1R). Early studies identified a role for SP and the NK1R in the digestive system, respiratory system, urogenital system, skin, nervous system, immune system, and tumors. More recently, SP and the NK1R have gained attention for their role in multiple viral infections, such as human immunodeficiency virus (HIV), HSV (herpes simplex keratitis), encephalomyocarditis virus (EMCV), respiratory syncytical virus (RSV), measles virus (MV), Epstein-Barr virus (EBV), enterovirus 71 (EV71) and so on. However, to date no human clinical assays using NK-1 receptor antagonists against viral infections have been developed, expect HIV. The use of aprepitant as anti-HIV therapy has been tested in human clinical trials, although this NK-1 receptor antagonist showed no significant anti-viral activity. NK-1 receptor antagonists merit further investigation as potential therapeutic antiviral agents. In this review, we update the involvement of the SP/NK-1 receptor system in viral infections, with the aim of providing insights for future anti-viral chemotherapy studies.

Keywords: Substance P, NK1-R, aprepitant, viral infection

Introduction

Substance P (SP) is an 11-amino acid peptide that belongs to the tachykinin family. Together with its receptors, SP play roles in a variety of pathophysiological processes. This article reviews the effects of SP and its receptors on viral infection.

SP, first detected in the horse brain and small intestine, promotes intestinal peristalsis and acts as an anti-hypertensive agent. It is the first member of the brain-gut peptide family, which is expressed in intestinal nerve cells, intestinal endocrine cells and canial nerve cells [1]. In 1970, 0.15 mg of pure SP was isolated from bovine hypothalamus tissue [2] and determined its amino acid sequence [3]. Later, biologically functional human SP was synthesized [4].

TAC1 (tachykinin-1) has two isomers, ATAC1 and BTAC1, which were initially cloned from the bovine striatum [5]. Both mRNAs are translated into the SP protein. The tachykinin family comprises 3 receptors, namely neurokinin receptor 1 (NK1-R), NK2-R and NK3-R, which are encoded by TACR1, TACR2, and TACR3, respectively. Nk1-R has the strongest affinity for SP, and NK1-R is commonly regarded as the SP receptor [6]. NK1-R has two isoforms: the full-length receptor and the truncated receptor [7].

SP is mainly synthesized by neurons, while some studies have shown that immune cells also express SP mRNA during the process of simian immunodeficiency virus (SIV)/human immunodeficiency virus (HIV) infection [8]. NK1-R is mainly expressed in T cells, B cells, monocytes/macrophages, NK cells, astrocytes and neurons [9].

After SP activates NK1-R in the human embryonic kidney 293 (HEK293) cell line, it causes rapid morphological changes, including the formation of cytoplasmic membrane vesicles [10], which are related to intracellular communication [11]. The binding of SP to NK1-R successively activates epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK) and extracellular signal-regulated protein kinase (ERK), and thus induces DNA syn-

thesis and cell proliferation [12, 13]. This mechanism partially mediates the ability of SP to promote the healing of colon epithelial cells [14]. Mucosal healing is related to the anti-apoptotic effects of SP, and the related signaling pathways include Janus kinase 2 (JAK-2) and phosphatidylinositol 3-kinase (PI3K)-mediated activation of the apoptotic molecule protein kinase B (PKB) [15]. The binding of SP to the NK1-R receptor in the U373MG cell line activates p38 and then induces the production of pro-inflammatory factors, including IL-6 and IL-8 [16]. SP also activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through Rho family kinases and protein kinase C δ (PKCδ), resulting in the production of IL-6, IL-8, and tumor necrosis factor-α (TNF-α) [17]. The binding of SP to NK1-R results in the expression of cyclooxygenase-2 (COX-2) and prostaglandin E2 [18]. SP activates a truncated NK1-R to inhibit IL-8 expression [19]. Treatments that block NK1-R glycosylation promote NK1-R endocytosis and inhibit IL-8 secretion [20]. Dorsal root ganglion neurons release SP, which mediates neurogenic inflammation and pain [6]. SP mainly mediates pain through two signaling pathways, the Ca^{2+}-phospholipid-dependent protein kinase pathway [21] and the cAMP-dependent protein kinase pathway [22].

The termination of SP signaling pathways relies on the degradation of SP by the brain-endorphin enzyme [23]. In addition, G-protein coupled receptor kinase 2 (GRK2), GRK3, and GRK5 cause Nk1-R phosphorylation and further desensitization [24].

The main pathophysiological functions of substance P have been determined. SP and its receptors play roles in the digestive system, respiratory system, urogenital system, skin, nervous system, immune system, and tumors. Stimulation of the intestinal nerves leads to NK1-R endocytosis in the intestinal muscle layer, which is associated with nerve conduction, SP release and NK1-R activation [25]. SP activates the non-selective calcium channel in the interstitial cells of Cajal, controlling the intestinal excitatory rhythm [26]. The expression of SP and NK1-R in the mucosal surface is increased by micro-cryptosporidium infection in the jejunum of rhesus monkeys, leading to the release of chloride ions and the inhibition of glucose absorption [27]. Three-nitrophenyl sulfonic acid activates the transient receptor potential cation channel subfamily V member 1, also called vanilloid receptor 1, promotes SP release and causes neurogenic colon inflammation [28]. NK1-R activation by spinal cord microglia and the p38 MAPK signaling pathway causes visceral hyperalgesia [29]. The binding of SP to NK1-R promotes the secretion of enzymes, nitric oxide, vasoactive intestinal peptide (VIP) and M2 muscarinic receptors in the mucous membrane of the respiratory tract [30]. SP and NK1-R play roles in airway allergic reactions [31]. SP causes bladder hypersensitivity by promoting intercellular adhesion molecule-1-mediated inflammation and reactive oxygen species production [32]. SP promotes the exudation of plasma by blood vessels, the degranulation of mast cells, the generation of reactive oxygen species and the expression of pro-inflammatory cytokines, chemokines, adhesion molecules and COX-2 in the bladder. SP promotes male sperm motility and participates in erection [33]. SP induces the production of pro-inflammatory factors [34] and nerve growth factors in skin keratinocytes, which regulates the regeneration of mucosal nerves [35]. SP promotes cutaneous nerve axon regeneration and wound healing [36]. SP plays a role in pain conduction by increasing glutamate release [37]. Strategies that reduce SP expression in the mouse spinal cord relieve cancer-induced pain [38]. NK1-R antagonists inhibit pain [39]. SP promotes the release of cytokines, chemokines, matrix metalloproteinases and reactive oxygen species from neutrophils, thereby promoting their phagocytosis of bacteria [40]. SP promotes the aggregation of dendritic cells and regulates the response of the lung to inhaled antigens [41]. SP promotes the growth of a variety of cancers, whereas NK1-R antagonists inhibit the growth of many cancer cells [42].

SP plays roles in the pathological processes of multiple viral infections and has both anti-viral and pro-viral effects. The application of its receptor antagonist as a treatment for viral infections has yielded promising preliminary results. The antiviral mechanisms of host proteins do not rely on viral DNA polymerase, which reduces the likelihood of viral drug resistance, and some host proteins penetrate the blood-brain barrier and the blood-ocular barrier. Therefore, we have reviewed the relevant stud-

ies, with the aim of providing insights for future anti-viral chemotherapy studies.

The role of substance P and its receptor in HIV infection

The role of SP in HIV infection

Cannabinoid receptor agonists inhibit glycoprotein 120 (Gp120)-mediated extracellular calcium influx; significantly reduce the permeability of human microvascular endothelial cells; inhibit zonula occludens-1 (ZO-1), claudin-5, and adhesion molecule-1 expression; inhibit human monocyte extravasation through the blood-brain barrier; and reduce the blood-brain barrier permeability. HIV-1 infection changes the structure and function of the blood-brain barrier, which may be related to HIV-related dementia. The mechanism may be mediated by the activation of brain endothelial cells by Gp120 and other viral components, leading to the increased blood-brain barrier permeability. Gp120 functions through an SP-mediated pathway to alter the expression of tight junction proteins and the permeability of cerebral endothelial cells. In addition, Gp120 directly promotes SP secretion, thus playing a role in HIV-related dementia. SP and Gp120 directly reduce the expression of ZO-1 and claudin-5 proteins, resulting in increased blood-brain barrier permeability [43]. SIV-infected apes have been used as a non-human primate model to study AIDS neuropathy. The expression levels of SP and NK1-R are increased in SIV encephalitis. Macrophages are the main cells expressing NK1-R, and all SIV-infected macrophages express NK1-R. SP-treated macrophages express C-C chemokine receptor type 5 (CCR5) and NK1-R on the cell membrane. An SP pretreatment increases SP and CCR5-mediated chemical chemotaxis, revealing the crosstalk between NK1-R and CCR5. SP promotes cellular transport through the blood-brain barrier, thereby accelerating the development of SIV encephalitis. According to a pathology study, NK1-R was expressed in the cingulate gyrus of patients who died of HIV encephalitis, which is similar to the results of SIV encephalitis [44]. The ability of HIV to infect macrophages is strengthened in the presence of cluster of differentiation 163 (CD163), and SP also increases HIV infection of macrophages by inducing CD163 expression. HIV displays greater infection efficiency in cells expressing CD163, and the efficiency of HIV infection is decreased in CD163 knockout macrophages. The binding of SP to NK1-R increases the intracellular calcium concentrations in mononuclear cells and the differentiation of monocytes to macrophages; moreover, macrophages expressing high levels of CD163 on the cell membrane are more sensitive to HIV infection [45]. SP promotes HIV infection in fetal brain cells that express full-length NK1-R receptors; the intracellular calcium concentration increases in cultured fetal brain cells after SP addition. The NK1-R receptor antagonist aprepitant prevents these effects, whereas the addition of SP to the cultured HIV-1-infected fetal brain cells increases HIV-1 expression. Cocktail therapy does not remove HIV-1 from the central nervous system, but aprepitant provides protection through the blood-brain barrier. NF-κB expression increases after SP binds to the full-length NK1-R, which promotes the expression of HIV/SIV in lymphocytes and monocytes and activates the latent HIV in hematopoietic progenitors [46].

Treatments that block NK1-R receptors inhibit HIV infection

Selective serotonin (5-hydroxy-trytamine) reuptake inhibitors and SP antagonists increase the innate immunity of NK cells infected with HIV. Meanwhile, the function of NK cells is significantly enhanced, which play a key role in inhibiting HIV infection. The plasma SP levels are elevated in HIV-infected patients. SP directly increases HIV replication in macrophages and T cells or indirectly increases HIV replication by altering beta-chemokine and receptor expression. Based on the results of in vitro experiments, the SP antagonist CP-96345 significantly increases the activity of NK cells in the peripheral blood of HIV-infected women [47]. Morphine withdrawal increases HIV infectivity in T lymphocytes by inducing SP expression. Sudden withdrawal and progressive withdrawal of morphine promotes HIV replication in human T cells with latent infections, and its mechanism is related to the increased expression of SP in peripheral blood lymphocytes and T cells. Opioid drugs significantly increase HIV infectivity. The SP receptor antagonist CP-96345 not only blocks morphine withdrawal caused by endogenous SP expression but also eliminates withdrawal-induced HIV replication

in T cells [48]. The SP receptor antagonist aprepitant prevents HIV-1 infection and exerts a synergistic effect in combination with other antiretroviral drugs. NK1-R receptor antagonists effectively penetrate the blood-brain barrier and thus play important roles in modulating the inflammatory response in the brain. In HIV-1-infected cultured peripheral blood mononuclear cells, the HIV-1 activity was determined by measuring the expression of the p24 antigen, and the joint application of aprepitant with ritonavir or saquinavir exerted synergistic effects. HIV-1 must be transmitted by CCR5, C-X-C motif chemokine receptor 4 (CXCR4) or both; the non-peptide SP receptor antagonists CP-96345 and aprepitant decrease CCR5 expression (CCR5 is the main HIV-1 receptor in macrophages) to further inhibit HIV infection of macrophages [49]. Phase 1 clinical trials of aprepitant as a treatment for HIV-1 infection was performed and the results showed that the number of CD4+ T cells expressing programmed death 1 (PD-1) and the concentrations of SP and soluble CD163 were decreased in the plasma of HIV-infected patients treated with aprepitant [50]. In this clinical trial, the dosage of aprepitant was 375 mg/day for 2 weeks, and the results showed that the drug was safe and had a significant biological effect, although an obvious anti-viral effect was not observed. Vapreotide is a synthetic analog of somatostatin that exerts analgesic effects by blocking the NK1-R receptors, and it inhibits the SP-induced increase in intracellular calcium concentrations, NF-κB activation, and IL-8 and MCP-1 generation. In vitro, vapreotide inhibits HIV infection of human macrophages derived from mononuclear cells; these effects disappeared when cells were pretreated with SP [51].

SP plays a role in the pathological process of herpes virus infection

The corneal sensitivity and SP levels decreased rapidly in a mouse model of herpes simplex keratitis (HSK), and the SP level recovered later than corneal sensitivity. SP does not participate in the disappearance of the blink reflex in the cornea, and SP could play an unknown role in the cornea [52]. Hamza Ma and his team [53] transformed primary cultured rat embryonic trigeminal ganglion cells with plasmids encoding HSV-1 latency-associated transcript (LAT) and the results showed that LAT increased the percentage of substance P-immunoreactive neurons by two thirds. Treatment with bone morphogenetic protein 7 (BMP-7) reversed the LAT-induced increase in SP expression in cultured cells, but the BMP-7 treatment did not affect the survival of the cultured neurons. LAT expression did not affect SP expression in the neurons in the dorsal root of the spinal cord. In the mouse HSK model, the SP level was higher in the corneas of mice with severe symptoms than in the corneas of mice with mild symptoms, and SP was mainly expressed in the corneal stroma and co-localized with β-III tubulin+ and IAb+ type cells. Based on these results, the SP content was 15-fold higher in the corneas of mice with severe symptoms than in the corneas of mice with mild symptoms. Corneas expressing high levels of SP also contained higher levels of pro-inflammatory factors and chemical chemokines, and subconjunctival injection of the SP receptor antagonist spantide II significantly reduced the corneal opacity and neovascularization in the clinical stage of the mouse HSK model. In addition, the severity of HSK lesions and the clearance rate of corneal virus were irrelevant. Although different virus clearance rates may lead to different severity of corneal symptoms, the corneal swabs collected at various time points failed to show a statistically significant difference in the amount of the virus present in the corneas between mice with mild symptoms and mice with severe symptoms. HSV-1 replication in the cornea is required to cause inflammation in the corneal stroma; however, the presence of the virus is not necessary to cause persistent lesions in the corneal tissue. SP regulates the inflammatory response to HSK in mice with differing degrees of symptom severity, but there were no statistically significant differences in the corneal levels of the virus in mice with different inflammatory responses [54].

The effect of SP on herpes virus infection is controversial

SP increased NO production in macrophages infected with HSV-1, but the effect decreased or disappeared after 24 hours [55]. SP increased the formation of HSV-1 plaques in a time dependent in macrophages treated with SP after HSV infection, and the effect was most evident 10 hours after SP treatment. However, pretreatment with SP before HSV-1

infection had no effect on the formation of HSV-1 plaques, but promoted the secretion of inflammatory factors in HSV-1-infected macrophages.

As shown in the study by Svensson and his team, the level of the SP expression was significantly increased in the vagina of HSV-2-infected mice. Furthermore, lack of SP signaling through the use of mice deficient in NK1R, revealed an important role for SP in the innate defense against HSV-2 [56]. The amount of HSV-2 virus in the genital tract of NK1-R-deficient mice infected with HSV-2 was significantly increased, and the progression of the disease was significantly accelerated, which was associated with an impaired NK cell activity in the genital tract. However, NK1-R deficiency had no effect on the animals' ability to mount a protective immune response to HSV-2 following vaccination with an attenuated virus. In conclusion, the present results indicated that, the SP-NK1-R signaling pathway plays a role in innate immunity against HSV-2 in mice. The average cytotoxicity of each NK cell in the vagina of NK1-R-/- mice decreased 8-fold, which impaired their ability to control viral replication. SP and NK1-R receptor blockers did not affect the ability of HSV-1 to form plaques, but 10-5 M SP reduced the plaque formation capacity by 42% [57]. Another research found that tachykinin 1 (TAC1) or TACR1 gene-deficient mice are more susceptible to the respiratory pathogen murine gamma herpes virus (MHV-68), but mice lacking both the TAC1 and NK1-R genes (NK1-/-xTAC1-/-) are more resistant to MHV-68 [58]. This may be due to a lack of feedback inhibition of other SP-binding receptors in these mouse. The study reconfirmed the roles of the TAC1 and TACR1 genes in controlling viral infections, but manifested the complexity of the mechanism of the peptide-receptor interactions. Deletion of the TAC1 gene and consequent lack of both SP and NKA leads to an increased susceptibility to MHV-68 infection, which may because of decreased inflammatory and specific cytotoxic T cell responses.

The effect of SP on viral myocarditis

Myocarditis, an inflammatory disorder of the heart, is most commonly caused by viral infections, such as coxsackie virus, echovirus, adenovirus and picornovirus. Viral myocarditis is characterized by cardiac inflammation and cardiomyocyte necrosis. Murine myocarditis caused by infection with encephalomyocarditis virus (EMCV) is a commonly used experimental model to study viral myocarditis. SP was required for the pathological process of EMCV infection in mice, and 8-week-old wild-type C57BL/6 mice infected with EMCV exhibited a 61-fold increase in SP levels [59]. EMCV-induced death, heart enlargement, heart inflammation, necrosis, apoptosis and hyperplasia were not observed in SP knockout mice. The SP expression level and the density of SP immunopositive cells decreased in the hearts of 4-week-old DBA/2 mice on the 6th day after EMCV infection [60]. SP levels in the hearts and ratio of heart weight to body weight of the mice was negatively correlated at 6 days, indicating that the level of SP in the heart is related to the severity of viral myocarditis and heart function. The plasma SP level was also lower on the 6th day and had decreased further by the 14th day after infection. The authors postulated that the reduced SP level may be related to the destruction of nerve fibers in the heart that contain SP. Because SP is distributed throughout the heart conduction system, the decrease in SP levels may affect cardiac electrical conduction and cause arrhythmia. However, in the above mentioned study by Robinson P and his team, the SP level increased in 8-week-old mice on the 14th day after EMCV infection, but myocardial necrosis and inflammation did not occur in SP knockout mice [59]. The differences between the two previous studies may be related to the different types and ages of mice used. The binding of SP to its receptor activates Rho A, leading to hypertrophy and apoptosis in cultured cardiomyocytes in vitro [61].

SP receptor antagonists protect against viral myocarditis

Pretreatment and posttreatment 1.2 mg/kg with aprepitant significantly reduced mortality, heart size, cardiomyocyte size and viral RNA content in mice infected with 50 PFU of EMCV [62]. However, pre- or posttreatment with the Rho inhibitor fasudil did not significantly affect the clinical and pathological manifestations in EMCV infected mice. The above results reveal that the effects of SP on cardiac-remodeling and dysfunction following ECMV infection is

mediated by its high affinity receptor, but not the Rho-A pathway, suggesting that SP-receptor antagonism may be a novel therapeutic-option for patients with viral-myocarditis.

The role of SP in respiratory syncytial virus infection

After the respiratory syncytial virus (RSV) infected the airway of the guinea pig, the balance of neuropeptide expression in lung neurons was influenced causing increased airway reactivity. The number of SP-positive neurons increased after RSV infection. As airway reactivity is maintained by the dynamic balance of two different components, excitatory components such as SP and inhibitory components such as NO and VIP, the stimulating inflammatory effects of SP were strengthened by RSV persistence. At the meantime, RSV infection-induced nerve growth factor (NGF) and neurotrophic factor expression, thus stimulating SP and NK1-R expression [63]. In another study, SP expression was markedly increased in mouse airway tissue after RSV infection, consistent with previous research. Prophylactic treatment with Sendide, a highly selective antagonist of the neurokinin-1 receptor inhibited the development of airway inflammation and airway hyperresponsiveness (AHR) in RSV-infected animals. Therapeutic treatment with calcitonin gene-related peptide abolished AHR in RSV-infected animals despite increased substance P levels and previously established airway inflammation [64]. Severe bronchitis in RSV-infected pediatric patients is associated with reduced airway IFN-γ and SP levels. Lower interferon-γ (IFN-γ) and SP concentrations were observed in the nasopharyngeal aspirates of infants in the oxygen-dependent and mechanical ventilation groups, and low IFN-γ and SP concentrations in the nasopharyngeal aspirates have been used as independent predictors of the severity of the disease [65].

Treatments that block SP reduce the apnea time caused by RSV infection

Apnea is a common complication in RSV-infected infants. In newly weaned rats, the duration of apnea was reduced after selective inhibition of NK1-R. Sensory nerve stimulation during RSV infection is related to the occurrence of apnea, and its mechanism is related to the binding of SP to NK1-R [66].

The role of SP and its receptor in measles virus (MV) infection

SP inhibits the replication of MV in cultured cells, and partially inhibits viral fusion to the cells of the hemolysis system. A single 0.6 mumol/l, SP treatment inhibited 50% of the MV infection, and the effect was completely reversible. The antiviral effect of SP is consistent with that of the 3- to 7-amino acid peptides synthesized by Schroeder C [67], which may have the same short sequence as the N-terminus of the fusion protein with the paramyxovirus (including the MV). SP blocks the spread of the MV between neurons. The use of NK1-R gene knockout or a drug blocking NK1-R reduces the susceptibility of mice to MV, suggesting that NK1-R plays a role in MV central nervous infection and transmission and may be a receptor or a co-receptor participating in the fusion of MV. In the experiment, most NK1-R-deficient newborn mice or normal adult mice treated with aprepitant survived the MV infection, with no sign of disease or virus replication in the central nervous system. NK1-R may be a receptor for MV in CD46+ neurons, and the SP-mediated blockade of measles infection may be associated with SP's competitive inhibition of NK-1 receptors [68].

The role of SP and its receptor in Epstein-Barr virus (EBV) infection

In the study published by Pierson DL and his team, the number of copies of EB virus in the saliva specimens of 32 astronauts during 10 air flights averaged 417/ml per air flight, which was significantly higher than the number of copies detected before the flights (40/ml) and after the flights (44/ml) [69]. In a study of 5 astronauts, the plasma SP concentrations were higher upon landing than before the flight. The observed elevated SP levels reduced cellular immunity and might have played a role in EB virus activation.

The role of SP and its receptor in enterovirus 71 (EV71) infection

The expression levels of the TAC1 and IL-17A genes are higher in mononuclear cells in the peripheral blood of rhesus monkeys infected with EV71 than in uninfected rhesus monkeys. EV71 is the main pathogen of hand, foot, and mouth disease (HFMD), and the TAC1 gene is

expressed at higher levels in the central nervous system than in the lungs. SP expression is significantly higher in the hypothalamus of rhesus monkeys infected with EV71 virus than in uninfected rhesus monkeys, suggesting that SP may play a role in EV71 virus infection [70].

Conclusion

SP is a widely distributed tachykinin and brain-gut peptide with important pathophysiological functions. SP and its receptors play important roles in viral infection, and clinical trials on the use of SP receptor antagonists to combat HIV infection have begun. Nerves are distributed in a wide range of tissues, and HSK is a type of keratitis that is closely related to the nervous system. Because SP is mainly synthesized by neurons, SP may play an important role in the course of HSK. A preliminary conclusion is that SP combats HSV-1 infection or regulates HSK symptoms; however, further studies of the function of SP and its receptor in HSK are needed.

Acknowledgements

The study was funded by National Natural Science fund of China (Fund Number: 8157-0820) and Huai'an Natural Science Research Project (Fund Number: HAB201738).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongwei Zhou, Department of Ophthalmology, Lianshui County People's Hospital, 6 Hongri Road, Lianshui, Huai'an 223400, Jiangsu, China. Tel: 86-15851785316; Fax: 86-517-82321458; E-mail: zhouhongwei@sjtu.org

References

[1] V Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. J Physiol 1931; 72: 74-87.

[2] Chang MM and Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J Biol Chem 1970; 245: 4784-4790.

[3] Chang MM, Leeman SE and Niall HD. Amino-acid sequence of substance P. Nat New Biol 1971; 232: 86-87.

[4] Tregear GW, Niall HD, Potts JT Jr, Leeman SE, Chang MM. Synthesis of substance P. Nat New Biol 1971; 232: 87-89.

[5] Nawa H, Hirose T, Takashima H, Inayama S and Nakanishi S. Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor. Nature 1983; 306: 32-36.

[6] Muñoz M and Coveñas R. Involvement of substance P and the NK-1 receptor in human pathology. Amino Acidsv 2014; 46: 1727-1750.

[7] Fong TM, Anderson SA, Yu H, Huang RR and Strader CD. Differential activation of intracellular effector by two isoforms of human neurokinin-1 receptor. Mol Pharmacol 1992; 41: 24-30.

[8] Ho WZ and Douglas SD. Substance P and neurokinin-1 receptor modulation of HIV. J Neuroimmunol 2004; 157: 48-55.

[9] Douglas SD, Lai JP, Tuluc F, Schwartz L and Kilpatrick LE. Neurokinin-1 receptor expression and function in human macrophages and brain: perspective on the role in HIV neuropathogenesis. Ann N Y Acad Sci 2008; 1144: 90-96.

[10] Meshki J, Douglas SD, Lai JP, Schwartz L, Kilpatrick LE and Tuluc F. Neurokinin 1 receptor mediates membrane blebbing in HEK293 cells through a Rho/Rho-associated coiled-coil kinase-dependent mechanism. J Biol Chem 2009; 284: 9280-9289.

[11] Chen P, Douglas SD, Meshki J and Tuluc F. Neurokinin 1 receptor mediates membrane blebbing and sheer stress-induced microparticle formation in HEK293 cells. PLoS One 2012; 7: e45322.

[12] Castagliuolo I, Valenick L, Liu J and Pothoulakis C. Epidermal growth factor receptor transactivation mediates substance P-induced mitogenic responses in U-373 MG cells. J Biol Chem 2000; 275: 26545-26550.

[13] Koh YH, Moochhala S and Bhatia M. Activation of neurokinin-1 receptors up-regulates substance P and neurokinin-1 receptor expression in murine pancreatic acinar cells. J Cell Mol Med 2012; 16: 1582-1592.

[14] Castagliuolo I, Morteau O, Keates AC, Valenick L, Wang CC, Zacks J, Lu B, Gerard NP and Pothoulakis C. Protective effects of neurokinin-1 receptor during colitis in mice: role of the epidermal growth factor receptor. Br J Pharmacol 2002; 136: 271-279.

[15] Koon HW, Zhao D, Zhan Y, Moyer MP and Pothoulakis C. Substance P mediates antiapoptotic responses in human colonocytes by Akt activation. Proc Natl Acad Sci U S A 2007; 104: 2013-2018.

[16] Fiebich BL, Schleicher S, Butcher RD, Craig A and Lieb K. The neuropeptide substance P activates p38 mitogen-activated protein kinase resulting in IL-6 expression independently from NF-kappa B. J Immunol 2000; 165: 5606-5611.

[17] Koon HW, Zhao D, Zhan Y, Simeonidis S, Moyer MP and Pothoulakis C. Substance P-stimulated interleukin-8 expression in human colonic epithelial cells involves protein kinase Cdelta activation. J Pharmacol Exp Ther 2005; 314: 1393-1400.

[18] Koon HW, Zhao D, Zhan Y, Rhee SH, Moyer MP and Pothoulakis C. Substance P stimulates cyclooxygenase-2 and prostaglandin E2 expression through JAK-STAT activation in human colonic epithelial cells. J Immunol 2006; 176: 5050-5059.

[19] Lai JP, Lai S, Tuluc F, Tansky MF, Kilpatrick LE, Leeman SE and Douglas SD. Differences in the length of the carboxyl terminus mediate functional properties of neurokinin-1 receptor. Proc Natl Acad Sci U S A 2008; 105: 12605-12610.

[20] Tansky MF, Pothoulakis C and Leeman SE. Functional consequences of alteration of N-linked glycosylation sites on the neurokinin 1 receptor. Proc Natl Acad Sci U S A 2007; 104: 10691-10696.

[21] Garcia M, Sakamoto K, Shigekawa M, Nakanishi S and Ito S. Multiple mechanisms of arachidonic acid release in Chinese hamster ovary cells transfected with cDNA of substance P receptor. Biochem Pharmacol 1994; 48: 1735-1741.

[22] Takeda Y, Blount P, Sachais BS, Hershey AD, Raddatz R and Krause JE. Ligand binding kinetics of substance P and neurokinin A receptors stably expressed in Chinese hamster ovary cells and evidence for differential stimulation of inositol 1,4,5-trisphosphate and cyclic AMP second messenger responses. J Neurochem 1992; 59: 740-745.

[23] Lu B, Figini M, Emanueli C, Geppetti P, Grady EF, Gerard NP, Ansell J, Payan DG, Gerard C and Bunnett N. The control of microvascular permeability and blood pressure by neutral endopeptidase. Nat Med 1997; 3: 904-907.

[24] Jorgensen R, Holliday ND, Hansen JL, Vrecl M, Heding A, Schwartz TW and Elling CE. Characterization of G-protein coupled receptor kinase interaction with the neurokinin-1 receptor using bioluminescence resonance energy transfer. Mol Pharmacol 2008; 73: 349-358.

[25] Harkins J, Roper M, Ham RG and Stewart JM. Biosynthesis of substance P in cultured mouse neuroblastoma and rat glioma cells. Brain Res 1978; 147: 405-409.

[26] d'antonio C, Wang B, McKay C and Huizinga JD. Substance P activates a non-selective cation channel in murine pacemaker ICC. Neurogastroenterol Motil 2009; 21: 985-e79.

[27] Hernandez J, Lackner A, Aye P, Mukherjee K, Tweardy DJ, Mastrangelo MA, Weinstock J, Griffiths J, D'Souza M, Dixit S and Robinson P. Substance P is responsible for physiological alterations such as increased chloride ion secretion and glucose malabsorption in cryptosporidiosis. Infect Immun 2007; 75: 1137-1143.

[28] Engel MA, Leffler A, Niedermirtl F, Babes A, Zimmermann K, Filipović MR, Izydorczyk I, Eberhardt M, Kichko TI, Mueller-Tribbensee SM, Khalil M, Siklosi N, Nau C, Ivanović-Burmazović I, Neuhuber WL, Becker C, Neurath MF and Reeh PW. TRPA1 and substance P mediate colitis in mice. Gastroenterology 2011; 141: 1346-1358.

[29] Bradesi S, Svensson CI, Steinauer J, Pothoulakis C, Yaksh TL and Mayer EA. Role of spinal microglia in visceral hyperalgesia and NK1R up-regulation in a rat model of chronic stress. Gastroenterology 2009; 136: 1339-1348, e1-e2.

[30] Rogers DF. Motor control of airway goblet cells and glands. Respir Physiol 2001; 125: 129-144.

[31] Hens G, Raap U, Vanoirbeek J, Meyts I, Callebaut I, Verbinnen B, Vanaudenaerde BM, Cadot P, Nemery B, Bullens DM, Ceuppens JL and Hellings PW. Selective nasal allergen provocation induces substance P-mediated bronchial hyperresponsiveness. Am J Respir Cell Mol Biol 2011; 44: 517-523.

[32] Candenas L, Lecci A, Pinto FM, Patak E, Maggi CA and Pennefather JN. Tachykinins and tachykinin receptors: effects in the genitourinary tract. Life Sci 2005; 76: 835-862.

[33] Tsai WH, Wu CH, Cheng CH and Chien CT. Ba-Wei-Di-Huang-Wan through its active ingredient loganin counteracts substance P-enhanced NF-κB/ICAM-1 signaling in rats with bladder hyperactivity. Neurourol Urodyn 2016; 35: 771-779.

[34] Song IS, Bunnett NW, Olerud JE, Harten B, Steinhoff M, Brown JR, Sung KJ, Armstrong CA and Ansel JC. Substance P induction of murine keratinocyte PAM 212 interleukin 1 production is mediated by the neurokinin 2 receptor (NK-2R). Exp Dermatol 2000; 9: 42-52.

[35] Burbach GJ, Kim KH, Zivony AS, Kim A, Aranda J, Wright S, Naik SM, Caughman SW, Ansel JC and Armstrong CA. The neurosensory tachykinins substance P and neurokinin A directly induce keratinocyte nerve growth factor. J Invest Dermatol 2001; 117: 1075-1082.

[36] Delgado AV, McManus AT and Chambers JP. Exogenous administration of substance P enhances wound healing in a novel skin-injury model. Exp Biol Med (Maywood) 2005; 230: 271-280.

[37] Lagerström MC, Rogoz K, Abrahamsen B, Lind AL, Olund C, Smith C, Mendez JA, Wallén-Mackenzie Å, Wood JN and Kullander K. A sensory subpopulation depends on vesicular gluta-

mate transporter 2 for mechanical pain, and together with substance P, inflammatory pain. Proc Natl Acad Sci U S A 2011; 108: 5789-5794.

[38] Cao R, Tu LF and Duan L. Effect of ginsenoside-Rd on allodynia and the expression of substance P and NK-1 receptor in the spinal dorsal horn of rats induced with spared nerve injury. Chinese Journal of Neuroanatomy 2011; 27: 8-13.

[39] Rupniak NM, Carlson E, Boyce S, Webb JK and Hill RG. Enantioselective inhibition of the formalin paw late phase by the NK1 receptor antagonist L-733,060 in gerbils. Pain 1996; 67: 189-195.

[40] Okaya T, Holthaus R, Kato A and Lentsch AB. Involvement of the neuropeptide substance P in lung inflammation induced by hepatic ischemia/reperfusion. Inflamm Res 2004; 53: 257-261.

[41] Kradin R, MacLean J, Duckett S, Schneeberger EE, Waeber C and Pinto C. Pulmonary response to inhaled antigen: neuroimmune interactions promote the recruitment of dendritic cells to the lung and the cellular immune response to inhaled antigen. Am J Pathol 1997; 150: 1735-1743.

[42] Munoz M, Covenas R, Esteban F and Redondo M. The substance P/NK-1 receptor system: NK-1 receptor antagonists as anti-cancer drugs. J Biosci 2015; 40: 441-463.

[43] Lu TS, Avraham HK, Seng S, Tachado SD, Koziel H, Makriyannis A and Avraham S. Cannabinoids inhibit HIV-1 Gp120-mediated insults in brain microvascular endothelial cells. J Immunol 2008; 181: 6406-6416.

[44] Vinet-Oliphant H, Alvarez X, Buza E, Borda JT, Mohan M, Aye PP, Tuluc F, Douglas SD and Lackner AA. Neurokinin-1 receptor (NK1-R) expression in the brains of SIV-infected rhesus macaques: implications for substance P in NK1-R immune cell trafficking into the CNS. Am J Pathol 2010; 177: 1286-1297.

[45] Tuluc F, Meshki J, Spitsin S and Douglas SD. HIV infection of macrophages is enhanced in the presence of increased expression of CD163 induced by substance P. J Leukoc Biol 2014; 96: 143-150.

[46] Schwartz L, Spitsin SV, Meshki J, Tuluc F, Douglas SD and Wolfe JH. Substance P enhances HIV-1 infection in human fetal brain cell cultures expressing full-length neurokinin-1 receptor. J Neurovirol 2013; 19: 219-227.

[47] Evans DL, Lynch KG, Benton T, Dubé B, Gettes DR, Tustin NB, Lai JP, Metzger D and Douglas SD. Selective serotonin reuptake inhibitor and substance P antagonist enhancement of natural killer cell innate immunity in human immunodeficiency virus/acquired immunodeficiency syndrome. Biol Psychiatry 2008; 63: 899-905.

[48] Wang X, Douglas SD, Peng JS, Zhou DJ, Wan Q and Ho WZ. An in vitro model of morphine withdrawal manifests the enhancing effect on human immunodeficiency virus infection of human T lymphocytes through the induction of substance P. Am J Pathol 2006; 169: 1663-1670.

[49] Manak MM, Moshkoff DA, Nguyen LT, Meshki J, Tebas P, Tuluc F and Douglas SD. Anti-HIV-1 activity of the neurokinin-1 receptor antagonist aprepitant and synergistic interactions with other antiretrovirals. AIDS 2010; 24: 2789-2796.

[50] Tebas P, Spitsin S, Barrett JS, Tuluc F, Elci O, Korelitz JJ, Wagner W, Winters A, Kim D, Catalano R, Evans DL and Douglas SD. Reduction of soluble CD163, substance P, programmed death 1 and inflammatory markers: phase 1B trial of aprepitant in HIV-1-infected adults. AIDS 2015; 29: 931-939.

[51] Spitsin S, Tuluc F, Meshki J, Ping LJ, Tustin IR and Douglas SD. Analog of somatostatin vapreotide exhibits biological effects in vitro via interaction with neurokinin-1 receptor. Neuroimmunomodulation 2013; 20: 247-255.

[52] Tullo AB, Keen P, Blyth WA, Hill TJ and Easty DL. Corneal sensitivity and substance P in experimental herpes simplex keratitis in mice. Invest Ophthalmol Vis Sci 1983; 24: 596-598.

[53] Hamza MA, Higgins DM and Ruyechan WT. Herpes simplex virus type-1 latency-associated transcript-induced immunoreactivity of substance P in trigeminal neurons is reversed by bone morphogenetic protein-7. Neurosci Lett 2007; 413: 31-35.

[54] Twardy BS, Channappanavar R and Suvas S. Substance P in the corneal stroma regulates the severity of herpetic stromal keratitis lesions. Invest Ophthalmol Vis Sci 2011; 52: 8604-8613.

[55] Yaraee R, Ebtekar M, Ahmadiani A, Sabahi F and Ghazanfari T. The effect of substance P on nitric oxide production by HSV-1 infected macrophages. Int Immunopharmacol 2007; 7: 135-139.

[56] Svensson A, Kaim J, Mallard C, Olsson A, Brodin E, Hökfelt T and Eriksson K. Neurokinin 1 receptor signaling affects the local innate immune defense against genital herpes virus infection. J Immunol 2005; 175: 6802-6811.

[57] Israel BA, Schultz KT and Murphy CJ. Lack of detectable interaction between HSV-1 and integrins or tachykinins. Intervirology 1998; 41: 132-134.

[58] Quinn JP, Kipar A, Hughes DJ, Bennett E, Cox H, McLaughlin L, Zimmer A, Hunt SP and Stewart JP. Altered host response to murine gammaherpesvirus 68 infection in mice lacking the tachykinin 1 gene and the receptor for substance P. Neuropeptides 2011; 45: 49-53.

[59] Robinson P, Garza A, Moore J, Eckols TK, Parti S, Balaji V, Vallejo J and Tweardy DJ. Substance P is required for the pathogenesis of EMCV infection in mice. Int J Clin Exp Med 2009; 2: 76-86.
[60] Wang Y, Shimada M, Xu Y, Hu D, Nishio R and Matsumori A. Role of substance P in viral myocarditis in mice. Heart Vessels 2010; 25: 348-352.
[61] Del Re DP, Miyamoto S and Brown JH. Focal adhesion kinase as a RhoA-activable signaling scaffold mediating Akt activation and cardiomyocyte protection. J Biol Chem 2008; 283: 35622-35629.
[62] Robinson P, Taffet GE, Engineer N, Khumbatta M, Firozgary B, Reynolds C, Pham T, Bulsara T and Firozgary G. Substance P receptor antagonism: a potential novel treatment option for viral-myocarditis. Biomed Res Int 2015; 2015: 645153.
[63] Tan YR, Yang T, Liu SP, Xiang Y, Qu F, Liu HJ and Qin XQ. Pulmonary peptidergic innervation remodeling and development of airway hyperresponsiveness induced by RSV persistent infection. Peptides 2008; 29: 47-56.
[64] Dakhama A, Park JW, Taube C, El Gazzar M, Kodama T, Miyahara N, Takeda K, Kanehiro A, Balhorn A, Joetham A, Loader JE, Larsen GL and Gelfand EW. Alteration of airway neuropeptide expression and development of airway hyperresponsiveness following respiratory syncytial virus infection. Am J Physiol Lung Cell Mol Physiol 2005; 288: L761-L770.
[65] Semple MG, Dankert HM, Ebrahimi B, Correia JB, Booth JA, Stewart JP, Smyth RL and Hart CA. Severe respiratory syncytial virus bronchiolitis in infants is associated with reduced airway interferon gamma and substance P. PLoS One 2007; 2: e1038.
[66] Sabogal C, Auais A, Napchan G, Mager E, Zhou BG, Suguihara C, Bancalari E and Piedimonte G. Effect of respiratory syncytial virus on apnea in weanling rats. Pediatr Res 2005; 57: 819-825.
[67] Schroeder C. Substance P, a neuropeptide, inhibits measles virus replication in cell culture. Acta Virol 1986; 30: 432-435.
[68] Makhortova NR, Askovich P, Patterson CE, Gechman LA, Gerard NP and Rall GF. Neurokinin-1 enables measles virus trans-synaptic spread in neurons. Virology 2007; 362: 235-244.
[69] Pierson DL, Stowe RP, Phillips TM, Lugg DJ and Mehta SK. Epstein-Barr virus shedding by astronauts during space flight. Brain Behav Immun 2005; 19: 235-242.
[70] Zhang Y, Yang E, Pu J, Liu L, Che Y, Wang J, Liao Y, Wang L, Ding D, Zhao T, Ma N, Song M, Wang X, Shen D, Tang D, Huang H, Zhang Z, Chen D, Feng M and Li Q. The gene expression profile of peripheral blood mononuclear cells from EV71-infected rhesus infants and the significance in viral pathogenesis. PLoS One 2014; 9: e83766.