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The inflammatory molecule sphingosine-1-phosphate is not effective to evoke or sensitize cough in naïve guinea pigs

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ABSTRACT

Sphingosine-1-phosphate (S1P) is an inflammatory mediator increased in the tissue in the number of inflammatory conditions. Preliminary data indicate that the vagal afferent neurons express several S1P receptors including $S1PR_{2-3}$. We therefore evaluated the hypothesis that S1P induces cough and/or enhances the cough evoked by other tussive stimuli (TRPA1 and TRPV1 activators) in naïve guinea pigs.

Inhalation of S1P in the concentrations of 0.1 mM and 1 mM did not evoke cough. Preinhalation and continuing inhalation of S1P (1 mM) during citric acid (0.2 M) challenge did not enhance citric acid-induced cough. Preinhalation of S1P and continuing inhalation during AITC (3 mM) challenge also did not enhance AITC-induced cough.

We conclude that S1P was not efficient to evoke cough in awake naïve guinea pigs. S1P was also not effective to sensitize the cough evoked by stimulation of TRPV1 and TRPA1 receptors. Nonetheless it cannot be excluded that S1P influences cough in the context of inflamed airways when the cough-mediating nerves undergo sensory neuroplasticity.

1. Introduction

Chronic cough is associated with significant comorbidities and decreased quality of life. During respiratory diseases there are more triggers for coughing in the airways. Many mediators have been identified that play significant roles in the initiation and progression of the disease. According to the current literature overview, there is not satisfactory treatment for such patients available; therefore every effort in searching for new promising therapies and targets in cough treatment is important. Pain treatments are being tested extensively in this field along with neuronal receptor antagonists (Canning et al., 2014; Dicpinigaitis et al., 2014; Belvisi and Birrell, 2017).

Membrane sphingolipids are known to be involved in the development and progression of many diseases including respiratory diseases and they are metabolized to form signalling molecules. Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite recognized as a regulator of many physiological and pathological processes (Maceyka et al., 2012; Natarajan et al., 2013; Aoki et al., 2016; Mohammed and Harikumar, 2017). S1P is formed by phosphorylation of sphingosine by two kinases, sphingosine kinase 1 and 2 (SphK1 and SphK2). On the other hand, S1P is regulated by its degradation mediated by S1P phosphatases and S1P lyases (Maceyka et al., 2012; Natarajan et al., 2013). Numerous agonists activate SphK1, including growth factors, hormones, pro-inflammatory cytokines, lipopolysaccharides, ligation of the IgE and IgG receptors, and many G-protein coupled receptors (GPCR) ligands (Maceyka et al., 2012) and this activation contributes to vast cellular processes and diverse signal pathways. S1P modulates immune function through binding to a set of G protein-coupled receptors termed $S1PR_{1-5}$ (Maceyka et al., 2012; Natarajan et al., 2013; Mohammed and Harikumar, 2017). After binding to specific receptors, S1P regulates a spectrum of biological functions including proliferation, cell survival, angiogenesis, barrier integrity or dysfunction, migration, immune and inflammatory function (Mohammed and Harikumar, 2017).

There is obvious evidence that overexpression of SphK1 and dysregulation of S1P correlates with progression of disease and SphK/S1P axis has been implicated in several diseases; therefore, it is often regarded as a potential therapeutic target (Mohammed and Harikumar, 2017). Interestingly, S1P levels were increased in the airways of asthmatic patients but not in control subjects. Moreover, S1P also regulates the function of airway smooth muscles during inflammation and airway remodelling (Ammit et al., 2001). Muscarinic receptor signalling leads to constriction of peripheral airways and the process involves the activation of SphKs and increase of intracellular Ca^{2+} levels (Mohammed

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and Harikumar, 2017). Other research study reports that systemic administration of S1P increased the airway resistance and cholinergic activity in whole mouse lung model (Roviezzo et al., 2015).

There is unresolved question whether stimulation of sphingosine receptors can affect afferent sensory nerves mediating the cough reflex. Recent study demonstrates a marked expression of receptors for inflammatory mediators in vagal nodose and jugular TRPV1-positive neurons in mouse lung including sphingosine-1-phosphate receptors. A subset of S1P receptors, namely $S1PR_{2-3}$ have been expressed apart from jugular neurons predominantly in intrapulmonary nodose neurons and the activation of S1P receptors by S1P agonists were shown in Ca assay given the fact that rapid increase in intracellular Ca^{2+} correlates with nociceptor activation (Wang et al., 2017). On the base of the knowledge that activation of S1P receptors can lead to sensory nerve activation and/or sensitization of vagal afferents nerves we evaluated the hypothesis that S1P stimulates vagal afferent nerves-induced cough and/or enhances the cough evoked by other tussive stimuli in guinea pigs tussive challenge model. To the best of our knowledge, S1P signalling cascade and its effects on the cough reflex has never been studied so far, thus we believed that this study can elucidate its potential role in the activation of airway defensive reflexes.

2. Material and methods

Male Hartley guinea pigs (weight 250–350 g, n = 35 in total) were used in this study. They were obtained from Velaz Ltd., Prague, Czech Republic. The animals were housed in institutional laboratory animal centre with air-conditioned room maintained at controlled room temperature of 22 ± 2 °C and 50 ± 5% relative humidity with alternating 12 h light/dark cycle, and were provided with standard food and tap water *ad libitum*. All animal experiments were approved by the Animal Ethical Committee at Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia and were performed in accordance with the institutional animal care guidelines and the statutes and legislation of Slovakia. After the quarantine, the animals were adapted to laboratory conditions and they were exposed to phosphate-buffered saline aerosol twice to eliminate the stress response during the first week of experiments.

2.1. Drugs

Sphingosine-1-phosphate (S1P) in concentration of 0.1 M–1 M (Cayman Chemical, USA), 0.2 M citric acid (Fisher Scientific, GmbH, Germany), 0.3 M allyl isothiocyanate (AITC) (Sigma-Aldrich, St. Louis, MO) and vehicles – phosphate-buffered saline (PBS) or bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO) dissolved in PBS were nebulized for period indicated for each experiment.

S1P was dissolved in PBS as a complex with bovine serum albumin (4 mg/ml) to a final concentration of 1 mM (0.1 M and 0.3 M) (van Koppen et al., 1996) and the solution was prepared by rapid stirring, sonicated and warmed at 45–60 °C water bath. TRPA1 agonist AITC was dissolved just prior to usage directly in PBS with constant stirring to final concentration of 3 mM. TRPV1 activator citric acid was dissolved in distilled water to final threshold concentration of 0.2 M. When we examined the effect of S1P, stock solution of S1P was added to solution of TRPA1 or TRPV1 activators. BSA as vehicle was used in order to assess the influence of the solvent on the efficacy of S1P.

2.2. Cough studies in conscious guinea pigs

The method for evaluation of cough has been validated previously (Brozmanova et al., 2002; Plevkova et al., 2004; Brozmanova et al., 2006; Brozmanova et al., 2008). The animals were individually placed in a double-chamber whole body plethysmograph (type 855, Hugo Sachs Electronik, March-Hugstetten, Germany). The head chamber was connected to the compressed air driven nebulizer (Metal Work, Pneumatic, Italy). A suction device set to balance the nebulizer output was connected to the head chamber to maintain constant airflow through the head chamber. Respiratory changes in the airflow were recorded using pneumotachograph Fleisch head connected to the head chamber and it was processed by an analog-digital converter (Biopack Systems, Inc, Model MP100, Santa Barbara, CA, USA). Data were obtained and analysed with the acquisition system ACQ Knowledge software installed in computer (Biopack Systems, Inc, Model MP100, Santa Barbara, CA, USA). Respiratory sounds including cough were recorded with a microphone from the head chamber connected to a preamplifier and MP3 recorder. The pneumotachograph and microphone output were simultaneously recorded for off-line analysis. Cough was detected by simultaneous analysis of pneumotachograph trace as the apparent expiratory airflow accompanied by the cough sound using software Sonic Visualizer in the recording.

In several sets of experiments, cough was induced by tussive stimuli using guinea pigs tussive challenge model. Cough challenge was performed using inhalation of S1P (1 mM) for 5 min with vehicle aerosol (0.4% BSA in PBS) pre-treatment during 5 min. Other experiments were designed to determine cough reflex sensitivity by inhalation of S1P (1 mM) aerosol for 5 min continually followed by inhalation of citric acid (0.2 M) aerosol for 5 min compared to inhalation of citric acid (0.2 M) aerosol alone during 5 min with vehicle (0.4% BSA in PBS) pretreatment to obtain control data for comparison. Finally, we provoked the cough reflex by inhalation of nebulized S1P (1 mM) followed by TRPA1 agonist AITC (3 mM) together with S1P (1 mM). In control experiments the vehicle was used instead of S1P. Each separated inhalation lasted 5 min (Table 1). The cough challenges were separated one week apart to avoid tachyphylaxis and to achieve a reproducible cough response.

2.3. Statistical analysis

For evaluation of data, non-parametric paired and non-paired comparisons tests were used as appropriate. Variables are presented as mean ± standard error of mean (SEM). $P < 0.05$ was regarded as statistically significant.

3. Results

Although we expected an increase in cough reflex sensitivity mediated by spingosine-1-phosphate as a lipid second messenger with mobilization of intracellular Ca^{2+} stores, our results have shown that inhalation of S1P in lower concentrations (0.1 mM and 1 mM) had no

Table 1
Experimental protocol of cough challenge.

Experiments Controls	1st inhalation (5 min)	2nd inhalation (5 min)
1. experiment	Vehicle – BSA (bovine serum albumin) in PBS	Sphingosine-1-phospate (S1P, 1 mM) (n = 24)
2a. experiment	Sphingosine-1-phospate (S1P, 1 mM)	S1P (1 mM) + Citric acid (0.2 M) (n = 12)
2b. control	Vehicle – BSA (bovine serum albumin) in PBS	Citric acid (0.2 M) (n = 21)
3a. experiment	Sphingosine-1-phospate (S1P, 1 mM)	S1P (1 mM) + AITC (3 mM) (n = 11)
3b. control	Vehicle – BSA (bovine serum albumin) in PBS	BSA in PBS + AITC (3 mM) (n = 12)