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Short communication

Sphingosine-1-phosphate augments agonist-mediated contraction in the bronchial smooth muscles of mice

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Abstract:
The effects of sphingosine-1-phosphate (S1P) on bronchial smooth muscle (BSM) contractility were investigated in naive mice. S1P had no effect on the basal tone of the isolated BSM tissues. However, in the presence of S1P (10^{-6} M), the BSM contractions induced by acetylcholine (ACh) and endothelin-1 (ET-1) were significantly augmented: both the ACh and ET-1 concentration-response curves were significantly shifted to the left. In contrast, the pretreatment with S1P had no effect on the contractions induced by high K^+ depolarization. It is thus possible that S1P augments BSM contraction induced by the activation of G protein-coupled receptors.

Key words:
sphingosine-1-phosphate (S1P), bronchial smooth muscle, airway hyperresponsiveness, asthma, mouse

Introduction

The dramatic increase in the number of asthma cases over the last decades is of great concern for public health worldwide [7]. Increased airway narrowing in response to nonspecific stimuli is a characteristic feature of human obstructive diseases, including bronchial asthma. This abnormality is an important sign of the disease, although the pathophysiological variations leading to hyperresponsiveness are unclear. It has been suggested that one of the factors that contributes to the exaggerated airway narrowing in asthmatics is an abnormality of the properties of airway smooth muscle [13, 20]. Rapid relief from airway limitation in asthmatic patients by β-stimulant inhalation may also suggest an involvement of augmented airway smooth muscle contraction in airway obstruction. Thus, for development of asthma therapy, it may be important to understand the changes in the contractile signaling of airway smooth muscle cells associated with the disease.

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that mediates diverse biological responses, including smooth muscle contraction [9, 12, 14, 16, 21]. Recently, the involvement of S1P in allergic bronchial asthma has been suggested [1, 8, 11, 15, 17–19]. Ammit and colleagues [1] first demonstrated that S1P levels are elevated in the airways of individuals with asthma after segmental allergen challenge. The finding that S1P can act as a chemotactic agent for eosinophils further suggests the involvement of S1P in the pathophysiology of asthma [18]. Indeed, inhalation of inhibitors of sphingosine kinase, which

produces S1P directly from sphingosine, attenuated antigen-induced airway inflammation in mice [15]. In addition, S1P might have an ability to cause airway hyperresponsiveness [8, 11, 17, 19]. Contrary to these observations, inhalation of S1P itself or FTY720, an S1P receptor agonist, prevented antigen-induced airway inflammation and hyperresponsiveness in mice [10]. Thus, the role of S1P in the development of asthma and airway hyperresponsiveness is still controversial.

In the present study, the effects of S1P on agonist-induced contraction were investigated in bronchial smooth muscles (BSMs) isolated from naive mice to determine whether S1P is involved in the augmented BSM contractility, one of the causes of airway hyperresponsiveness in asthmatics.

Materials and Methods

Animals

Male BALB/c mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and housed in a pathogen-free facility. All animal experiments were approved by the Animal Care Committee of Hoshi University (Tokyo, Japan).

Functional studies

Mice were sacrificed by exsanguination from the abdominal aorta under urethane anesthesia (1.6 g/kg, *ip*; Sigma Aldrich, St. Louis, MO, USA), and the airway tissues from the larynx to the lungs were immediately removed. A segment of approximately 3 mm in length of the left main bronchus (~0.5 mm diameter) was isolated, and the epithelium was removed by gently rubbing with sharp tweezers [6]. The resultant tissue ring preparation was then suspended in a 5 ml organ bath using two stainless-steel wires (0.2 mm diameter) passed through the lumen. For all tissues, one end was fixed to the bottom of the organ bath, and the other was connected to a force-displacement transducer (TB-612T, Nihon Kohden) for the measurement of isometric force. A resting tension of 0.5 g was applied. The buffer solution contained modified Krebs-Henseleit solution with the following composition (mM); NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 10.0. The buffer solution was maintained at 37°C and oxygenated with 95% O_2 – 5% CO_2. The BSM responsiveness to agonists and isotonic high K^+ solutions was measured as previously described [2]. The high K^+ stimulation was carried out in the presence of atropine and indomethacin (both at final concentrations of 10^{-6} M) [2]. The tissues were also treated with S1P (10^{-6} M final concentration; Cayman Chemical Co., Ann Arbor, MI, USA) or its vehicle (methanol; at a final concentration of 1%) 30 min prior to the application of the stimulant.

Statistical analyses

All data were expressed as the mean with SE. The statistical significance of difference was determined using an unpaired Student's *t*-test or two-way analysis of variance (ANOVA) with a *post-hoc* Bonferroni/Dunn test (StatView for Macintosh ver. 5.0, SAS Institute, Inc., NC). A value of $p < 0.05$ was considered significant.

Results and Discussion

As described above, the involvement of S1P in allergic bronchial asthma has been suggested by the fact that S1P levels are elevated in the airways of asthmatic individuals [1]; however, its physiological and/or pathophysiological roles in the airways are not fully understood. To determine the effects of S1P on BSM contractility, the BSM tissues isolated from naive mice were incubated with S1P (10^{-6} M) for 30 min. Under the experimental conditions currently used, S1P had no effect on basal BSM tone (data not shown). In agreement with this finding, Roviezzo and colleagues [19] also revealed that S1P itself had no significant effect on the basal tone of the BSMs isolated from BALB/c mice. In contrast, Kume and colleagues [11] reported a marked contraction from basal tone induced by S1P (10^{-6} M) in tracheal smooth muscles isolated from guinea pigs. Some species and/or regional differences may be involved in the difference in the S1P response of airway smooth muscles. In addition, some tissue-dependent differences in the contractile activity of S1P might also exist because S1P itself has the ability to contract smooth muscles of the rabbit stomach [9],

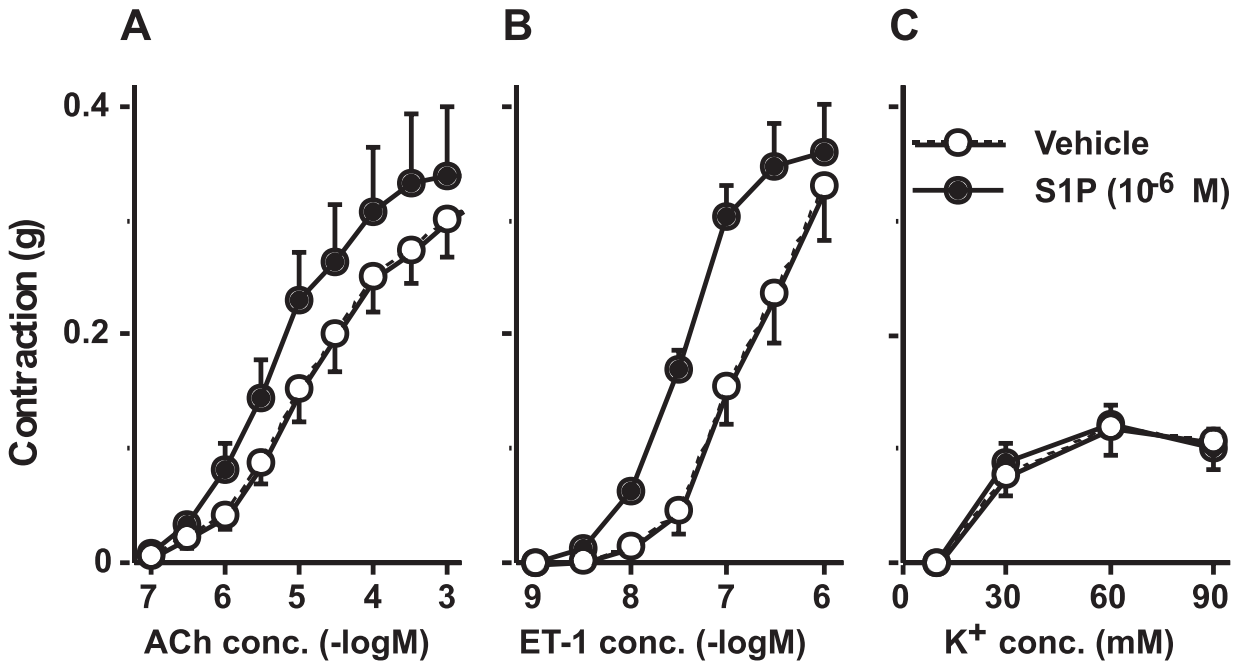

Fig. 1. Effects of *in vitro* treatment with sphingosine-1-phosphate (S1P) on bronchial smooth muscle responsiveness to acetylcholine (ACh; **A**), endothelin-1 (ET-1; **B**), and isotonic high K^+ depolarization (in the presence of atropine and indomethacin, both 10^{-6} M; **C**) in naive mice. Smooth muscle preparations were isolated from the left main bronchi and were pretreated with S1P (10^{-6} M) or its vehicle (1% methanol) 30 min prior to the application of the stimulant. Each point represents the mean with the SEM from 5 different animals. * $p < 0.05$ and ** $p < 0.001$ *vs.* the vehicle group by Bonferroni/Dunn's test. Note that both of the concentration-response curves for ACh (**A**) and ET-1 (**B**) were significantly shifted to the left in the S1P-treated group ($p < 0.05$, respectively, by two-way ANOVA)

mouse thoracic aorta [12], dog cerebral artery [14] and cat esophagus [21] and to contract cultured human coronary artery smooth muscle cells [16] in the concentration range of 10^{-9} to 10^{-6} M.

Figure 1 shows the contractile responsiveness to acetylcholine (ACh; Fig. 1A), endothelin-1 (ET-1; Fig. 1B), and high K^+ depolarization (Fig. 1C) of BSMs isolated from normal mice. As shown in Figure 1A, the ACh concentration-response curve of BSMs treated with S1P (10^{-6} M) was significantly shifted to the left: the pD_2 ($-\log EC_{50}$) value of the S1P-treated BSMs (5.39 ± 0.35) was significantly greater than that of the vehicle-treated muscles (4.89 ± 0.13, $p < 0.05$). Similarly, the ET-1 responsiveness was significantly augmented by the S1P treatment (Fig. 1B): the pD_2 value of the S1P-treated BSMs (7.46 ± 0.07) was significantly greater than that of the vehicle-treated muscles (6.89 ± 0.05, $p < 0.001$). In contrast, S1P had no effect on the BSM responsiveness to high K^+ depolarization (Fig. 1C). These findings suggest that S1P has an ability to augment the contraction mediated by G protein-coupled receptors (GPCRs). In human coronary artery smooth muscle cells, S1P induced a contraction, which was inhibited by a Rho-kinase inhibitor, Y-27632 [16]. In rabbit gastric smooth muscle cells, S1P caused an activation of Rho-kinase [9]. Our previous studies demonstrated that the RhoA/Rho-kinase pathway is activated both by ACh and ET-1, but not by high K^+ depolarization, in rodent BSMs [3–6]. Taken together, these data suggest that it is possible that S1P augments BSM contraction, probably by augmenting the agonist-induced RhoA/Rho-kinase-mediated Ca^{2+} sensitization in mice. This hypothesis might also be supported by the report that the S1P-mediated augmentation of methacholine-induced contraction was abolished by Y-27632 in tracheal smooth muscles of guinea pigs [11].

In summary, the *in vitro* treatment with S1P augmented the contraction induced by ACh and ET-1 in BSMs of mice. Further detailed studies are needed to elucidate the role of S1P in the pathogenesis of allergic bronchial asthma.

Conflict of interest:
None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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