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Analysis of responses to the TRPV4 agonist GSK1016790A in the pulmonary vascular bed of the intact-chest rat

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Pankey EA, Zsombok A, Lasker GF, Kadowitz PJ. Analysis of responses to the TRPV4 agonist GSK1016790A in the pulmonary vascular bed of the intact-chest rat. Am J Physiol Heart Circ Physiol 306: H33–H40, 2014. First published November 1, 2013; doi:10.1152/ajpheart.00303.2013.—The transient receptor potential vanilloid 4 (TRPV4) channel is a nonselective cation channel expressed on many cell types, including the vascular endothelium and smooth muscle cells. TRPV4 channels play a role in regulating vasomotor tone and capillary permeability. The present study was undertaken to investigate responses to the TRPV4 agonist GSK1016790A on the pulmonary and systemic vascular beds in the rat. Intravenous injection of GSK1016790A at doses of 2–10 μg/kg produced dose-dependent decreases in systemic arterial pressure, small decreases in pulmonary arterial pressure, and small increases in cardiac output, and responses were not altered by the cyclooxygenase inhibitor miconazole. Injection of GSK1016790A at a dose of 12 μg/kg iv produced cardiovascular collapse that was reversible in some animals. GSK1016790A produced dose-related increases in pulmonary and systemic arterial pressure when baseline tone in the pulmonary vascular bed was increased with U-46619. After treatment with the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME), the results of this study show that GSK1016790A has potent vasodilator activity in the systemic vascular bed and modest pulmonary vasodilator activity under resting tone conditions. Pulmonary vasodilator responses to GSK1016790A were enhanced when tone was increased with U-46619 and converted to a biphasic response with a potent vasoconstrictor component, which was antagonized by isradipine when NOS was inhibited with L-NAME. Systemic vasodilator responses to GSK1016790A were enhanced by L-NAME treatment. Pulmonary and systemic vasoconstrictor responses to GSK1016790A were attenuated by the TRPV4 antagonist GSK2193874. The present data indicate that responses to the TRPV4 agonist are mediated differently in pulmonary and systemic vascular beds when NOS is inhibited with L-NAME.

METHODS

The Institutional Animal Care and Use Committee of the Tulane University School of Medicine approved the experimental protocol used in this study, and all procedures were conducted in accordance with institutional guidelines. In these experiments, adult male Sprague-Dawley rats (Charles Rivers) weighing 333–415 g were anesthetized with Inactin (100 mg/kg ip, Sigma-Aldrich) and were placed in the supine position on an operating table. Supplemental doses of Inactin were administered intraperitoneally to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of polyethylene (PE)-240 tubing to maintain a patent airway. Animals spontaneously breathed room air. A femoral artery was catheterized with PE-50 tubing for measurements of systemic arterial pressure. The left jugular and femoral veins were catheterized with PE-50 tubing for intravenous injections and infusions of agents. For pulmonary arterial pressure measurements, a
specially designed 3-Fr single lumen catheter with a curved tip and with a radiopaque marker was passed from the right jugular vein and into the main pulmonary artery under fluoroscopic guidance (Picker-Surveyor Fluoroscope) (14). Pulmonary and systemic arterial pressures were measured with Nemic Perceptor DT transducers (Boston Scientific), digitized by a Biopac MP100 data-acquisition system (Biopac Systems), continuously recorded, and stored on a Dell PC. Cardiac output was measured by the thermodilution technique with a Cardiomax II computer (Columbus Instruments). A known volume (0.2 ml) of room temperature 0.9% NaCl solution was injected into the jugular vein catheter with the tip near the right atrium, and changes in blood temperature were detected by a 1.5-Fr thermistor microprobe catheter (Columbus Instruments) positioned in the aortic arch from the left carotid artery. Indicator dilution curve data were stored on the PC.

Experiments. Each experimental series was carried out in a separate group of animals. Bolus injections of the TRPV4 agonist were given in a fixed volume of 150 μl. The order of agonist injection was randomized, and sufficient time was permitted between agonist injections for pressures to return to control values.

In the first set of experiments, the effects of intravenous injections of the TRPV4 agonist GSK1016790A (Sigma-Aldrich) at doses of 2, 4, 6, 8, and 10 μg/kg and, in some experiments, responses to 12 μg/kg iv of the agonist on pulmonary and systemic arterial pressures as well as cardiac output were investigated in the anesthetized intact-chest rat under baseline conditions.

In the second set of experiments, responses to intravenous injections of the TRPV4 agonist GSK1016790A at doses of 2, 4, 6, 8, and 10 μg/kg when pulmonary arterial pressure was increased to ~30 mmHg by continuous intravenous infusion of the thromboxane receptor agonist U-46619 were investigated. After an initial high priming rate (400 ng/min), the U-46619 infusion was adjusted (150–250 ng/min) to maintain pulmonary arterial pressure at ~30 mmHg.

In the third set of experiments, the effect of the NOS inhibitor l-NAME (30 mg/kg iv) on responses to intravenous injections of GSK1016790A at doses of 2, 4, 6, 8, and 10 μg/kg in pulmonary and systemic vascular beds were investigated to determine the role of endogenous NO production in modulating responses to the TRPV4 agonist.

In the fourth set of experiments, the effect the cytochrome P-450 inhibitor miconazole (50 mg/kg iv) and of the cyclooxygenase inhibitor sodium meclofenamate (5 mg/kg iv) on responses to intravenous injections of a midrange dose of GSK1016790A (6 μg/kg iv) were investigated to determine the role of cytochrome P-450 and cyclooxygenase products in mediating or modulating responses to GSK1016790A.

In the fifth set of experiments, the effect of simultaneous administration of the L-type Ca\(^{2+}\) entry antagonist isradipine (1 μg/gk iv) on responses to the TRPV4 agonist GSK1016790A (6 μg/gk iv) in l-NAME-treated animals was investigated to provide information on the Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels.

In the sixth set of experiments, the effect of the TRPV4 agonist GSK2193874 at a dose of 300 μg/kg iv on responses to 6 μg/kg GSK1016790A were investigated under baseline and elevated tone conditions and in l-NAME-treated animals. The dose of GSK2193874 was determined from the literature and from a pilot study (18).

Immunohistochemical localization of TRPV4 was performed in a similar manner to that previously described (16, 19). Rats were anesthetized with ketamine and xylazine (0.99 mg/kg) and perfused transcardially with 0.15 M sodium phosphate buffer (pH 7.4) followed by 4% paraformaldehyde in 0.15 M sodium phosphate buffer. The lung was removed, postfixed for overnight in the same fixative, rinsed in 0.01 M PBS (pH 7.4), immersed in 30% sucrose in PBS until they equilibrated, and sectioned at 20 μm with a cryostat. After several rinses in PBS, sections were incubated in PBS with 10% normal goat serum and 0.5% Triton X-100 (1 h at room temperature) and then immersed in rabbit anti-TRPV4 antibody (no. 3926o, Abcam) in PBS or anti-α-smooth muscle actin antibody (1:100, 48 h at 4°C) with 0.1% Triton X-100 and 1% normal goat serum. After several rinses in PBS, sections were treated with a fluorescence-conjugated (Alexa fluor 488 or 594, Molecular Probes, Eugene, OR) goat anti-rabbit secondary antibody (IgG, 1:200, 1 h at room temperature) followed by more rinsing in PBS. Sections were air dried, covered in an antioxidant medium (Vectorshield, Vector Laboratories), and coverslipped.

Representative 20-μm-thick tissue sections were serially taken for immunohistochemistry and histological evaluation. To determine the immunohistochemical localization of TRPV4, a Nikon Eclipse 50i light microscope and digital camera were used.

Drugs. The TRPV4 agonist GSK1016790A (Sigma-Aldrich) and the TRPV4 antagonist GSK2193874 were prepared in 1% DMSO and 20% Captisol (sulfolubity ether-β-cycloexetrin, CyDex, Lenexa, KS) and saline. U-46619 (Cayman Chemical) was dissolved in 95% ethyl alcohol and diluted in 0.9% NaCl solution. l-NAME, sodium meclofenamate, sodium nitroprusside, and phenylephrine (Sigma-Aldrich) were dissolved in 0.9% NaCl. Miconazole (Sigma-Aldrich) was dissolved in DMSO. Isradipine (Sigma-Aldrich) was dissolved in 1% Tween 80 and 0.9% NaCl.

Statistics. Hemodynamic data are expressed as means ± SE and were analyzed using paired and group t-tests and ANOVA with repeated measures. The criterion used for statistical significance was P < 0.05.

RESULTS

Responses to GSK1016790A. GSK-1016790A is a recently discovered small-molecule potent activator of TRPV4 channels in a variety of biological systems (22). In the present study responses, to intravenous injections of the TRPV4 agonist were investigated in the anesthetized rat. Intravenous injections of GSK1016790A at doses of 2, 4, 6, 8, and 10 μg/kg produced dose-related decreases in systemic arterial pressure, small decreases in pulmonary arterial pressure, and small increases in cardiac output under baseline tone conditions (Fig. 1). The threshold dose of 2 μg/kg iv was determined in pilot studies. and smaller doses did not produce a significant change in systemic arterial pressure. Intravenous injection of the vehicle for GSK101690A had no significant effect on pulmonary and systemic arterial pressure or on cardiac output. Intravenous injection of the 12 μg/kg dose of the TRPV4 agonist produced a large decrease in systemic arterial pressure averaging 47 ± 4 mmHg, and injection of GSK1016790A at doses larger than 10 μg/kg was lethal in four of six animals. Vascular resistance in the systemic and pulmonary vascular beds was calculated as follows: resistance = (arterial pressure/cardiac output). When GSK1016790A was injected at a dose of 10 μg/kg iv, systemic arterial pressure decreased from 98 to 51 mmHg and cardiac output increased from 108 to 118 ml/min, so that systemic vascular resistance decreased 56%. Systemic vascular resistance was decreased 11–56% at doses of 2–10 μg/kg iv of GSK1016790A. Pulmonary vascular resistance was decreased 11–14% at doses of 2–10 μg/kg iv of the TRPV4 agonist under baseline tone conditions.

Intravenous injection of GSK1016790A at a dose of 12 μg/kg iv produced a cardiovascular collapse response and produced mortality in four of six rats. There was a marked decrease in systemic arterial pressure averaging 67 ± 5 mmHg and an acute increase in pulmonary arterial pressure followed by a sharp decrease in pressure in response to intravenous injection of the 12 μg/kg dose of the TRPV4 agonist. In animals surviving the collapse response, systemic and pulmonary arterial pressure and cardiac output were restored...
to baseline values within 10 min after the injection of GSK1016790A without the benefit of resuscitation. These data indicate that the TRPV4 agonist GSK1016790A has marked vasodilator activity in systemic and pulmonary vascular bed of the rat when vasoconstrictor tone is elevated. These data also demonstrate that a recently described cardiovascular collapse response, which in the present study was reversible in some rats, was observed in response to intravenous injection of GSK1016790A (22).

**Effect of elevated tone.** The analysis of vasodilator responses in the pulmonary vascular is difficult when baseline tone is low; therefore, responses to GSK1016790A were investigated when vasoconstrictor tone in the pulmonary vascular bed was increased to a high steady level by the thromboxane (TP) receptor agonist U-46619. Intravenous infusion of the TP receptor agonist increased pulmonary arterial pressure to ~30 mmHg (Table 1). When pulmonary arterial pressure was increased to a high steady value, intravenous injection of the TRPV4 agonist at doses of 2–10 μg/kg iv produced dose-related decreases in pulmonary and systemic arterial pressure and produced small increases in cardiac output (Fig. 2). Pulmonary vascular resistance was decreased by 18–36%, whereas systemic vascular resistance was decreased 8–69%, in response to intravenous injections of GSK1016790A at doses of 2–10 μg/kg iv (Table 1). The results of these experiments indicate that the TRPV4 agonist has potent vasodilator activity in the systemic and pulmonary vascular beds when pulmonary arterial pressure is increased to ~30 mmHg with infusion of the thromboxane (TP) receptor agonist U-46619.

### Table 1. Effect of U-46619 on systemic and pulmonary arterial pressure and cardiac output

<table>
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<tr>
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<th>Systemic Arterial Pressure, mmHg</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
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<tbody>
<tr>
<td>Control</td>
<td>100 ± 4</td>
<td>20 ± 1</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>U-46619</td>
<td>103 ± 3</td>
<td>31 ± 1*</td>
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Values are means ± SE; n = 14. *P < 0.05 compared with control.
vasoconstrictor tone is increased to a high steady level with U-46619.

Effect of l-NAME. It has been previously reported that the TRPV4 agonist increases intracellular Ca\(^{2+}\) concentration and activates endothelial NOS in endothelial cells (1, 22). The role of NOS and NO in mediating or modulating cardiovascular responses to the TRPV4 agonist GSK1016790A were investigated in experiments with the NOS inhibitor l-NAME; these data are shown in Fig. 3. The administration of l-NAME at a dose of 50 mg/kg iv produced significant increases in pulmonary and systemic arterial pressure and a significant decrease in cardiac output (Table 2). When pulmonary and systemic arterial pressures had risen to a high steady value after treatment with the NOS inhibitor, responses to intravenous injections of the TRPV4 agonist GSK1016790A were investigated. In these experiments, intravenous injections of GSK1016790A at doses of 2, 4, and 6 \(\mu\)g/kg produced dose-dependent decreases in systemic arterial pressures, small increases in cardiac output, and large dose-dependent increases in pulmonary arterial pressure followed by a small decrease in pulmonary arterial pressure (Figs. 3 and 4). A record from an experiment illustrating the increase in pulmonary arterial and the decrease in systemic arterial pressure in response to intravenous injections of GSK1016790A at a dose of 4 \(\mu\)g/kg iv is shown in Fig. 4, and summary data are shown in Fig. 3. The increase in pulmonary arterial pressure and decreases in systemic arterial pressure in response to the TRPV4 agonist were large in magnitude and rapid in onset (Fig. 3). There was a small secondary decrease in pulmonary arterial pressure in most experiments. The largest increase in systolic pressure in the pulmonary artery in response to the TRPV4 agonist was 40 mmHg, with an increase in diastolic pressure of 19 mmHg, and mean pulmonary arterial pressure was increased to 46 mmHg. The decreases in systemic arterial pressure in response to the TRPV4 agonist were greatly enhanced by treatment with l-NAME. The decreases in systemic arterial pressure in response to GSK1016790A (2, 4, and 6 \(\mu\)g/kg iv) in l-NAME-treated animals were large (10, 45, and 70 mmHg) and were significantly greater than responses to these doses of the TRPV4 agonist in control and U-46619-infused animals. Systemic vascular resistance decreased by 22%, 44%, and 64%. Intravenous injection of the TRPV4 agonist at doses of 2, 4, and 6 \(\mu\)g/kg increased pulmonary vascular resistance 7%, 34%, and 50%, respectively, in l-NAME-treated animals. The secondary phase of the pulmonary arterial pressure response to the TRPV4 agonist was characterized by small decreases in pulmonary arterial pressure compared with baseline values. Injection of GSK1016790A at doses of 8

### Table 2. Effect of l-NAME on systemic and pulmonary arterial pressure and cardiac output

<table>
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<tr>
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<th>Systemic Arterial Pressure, mmHg</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 ± 3</td>
<td>19 ± 1</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>l-NAME</td>
<td>145 ± 4*</td>
<td>30 ± 1*</td>
<td>59 ± 5*</td>
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Values are means ± SE; \(n = 16\). l-NAME, N\(^{n}\)-nitro-l-arginine methyl ester. *\(P < 0.05\) compared with control.
μg/kg iv or greater in L-NAME-treated animals resulted in cardiopulmonary collapse and was lethal in five of six animals. The results of experiments in L-NAME-treated rats indicate that the TRPV4 agonist GSK1016790A had potent pulmonary vasoconstrictor activity and potent systemic vasodilator activity when NOS was inhibited with L-NAME. These data indicate that NOS and NO have a marked and opposite modulatory role on vasomotor responses to activation of TRPV4 receptors in pulmonary and systemic vascular beds in the rat.

Effect of isradipine on responses to GSK1016790A in L-NAME-treated animals. The role of Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels in mediating the increase in pulmonary arterial pressure in response to intravenous injection of GSK1016790A in L-NAME-treated rats was investigated using the Ca$^{2+}$ antagonist isradipine. Coadministration of GSK1016790A (6 μg/kg) and isradipine (1 μg/kg) resulted in significantly smaller increases in pulmonary arterial pressure and a larger decrease in systemic arterial pressure (Fig. 3B). The results with isradipine may be interpreted to suggest that increased Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels may have a role in mediating the increase in pulmonary arterial pressure in response to GSK1016790A in L-NAME-treated rats.

Effect of the TRPV4 antagonist GSK2193874 on responses to GSK1016790A. The effect of the TRPV4 antagonist GSK2193874 on responses to GSK1016790A were investigated. After treatment with the TRPV4 antagonist at a dose of 300 μg/kg iv decreases in systemic and pulmonary arterial pressure in response to intravenous injection of GSK1016790A (6 μg/kg) were decreased significantly under baseline tone conditions and in U-46619-infused animals (Fig. 5, A and B). The decrease in systemic arterial pressure and the increase in pulmonary arterial pressure in response to intravenous injection of GSK1016790A (6 μg/kg) in L-NAME-treated animals were significantly attenuated by the TRPV4 antagonist GSK2193874 (Fig. 5C). The TRPV4 antagonist had no significant effect on vasodilator responses to sodium nitroprusside or vasoconstrictor responses to phenylephrine (data not shown). Intravenous injection of the TRPV4 antagonist at a dose of 300 μg/kg had no significant effect on baseline pressures in pulmonary or systemic vascular beds. The data with the TRPV4 receptor antagonist indicate that pulmonary and systemic vasomotor responses to GSK1016790A are mediated by the activation of TRPV4 receptors.

Fig. 5. Bar graphs showing the effect of the TRPV4 antagonist GSK2193874 at a dose of 300 μg/kg iv on changes in pulmonary and systemic arterial pressure and cardiac output in response to intravenous injection of the TRPV4 agonist GSK1016790A (6 μg/kg iv) under baseline tone conditions (A), when pulmonary tone was elevated with U-46619 (B), and after treatment with L-NAME (50 mg/kg iv; C). n is the number of experiments. *P < 0.05 compared with control.
**Effect of meclofenamate and miconazole.** Effects of the cyclooxygenase inhibitor sodium meclofenamate and the cytochrome P-450 epoxygenase inhibitor miconazole on responses to GSK1016790a were investigated, and these data are shown in Fig. 6. Intravenous injection of sodium meclofenamate at a dose of 5mg/kg or miconazole at a dose of 50 mg/kg had no significant effect on the decreases in pulmonary or systemic arterial pressure or increases in cardiac output in response to intravenous injections of a midrange dose (6μg/kg) of the TRPV4 agonist GSK1016790A (Fig. 6).

**Immunohistochemical localization of TRPV4 receptors.** Expression of the TRPV4 channel was investigated in the rat lung using fluorescent immunostaining, and these data are shown in Fig. 7. TRPV4 immunoreactivity was observed in small pulmonary arteries, and a representative image is shown in Fig. 7A. The asterisk in Fig. 7A indicates the vessel lumen, and white arrows indicate positive TRPV4 immunofluorescent staining. Strong immunoreactivity was observed in the airway epithelium, and TRPV4 immunoreactivity was detected in the area of the interface of intima and medial layers of medium-sized pulmonary arteries (Fig. 7A). Smooth muscle actin was used to label the vessel walls (Fig. 7B). To test the specificity of TRPV4 staining, we performed staining experiments without the primary antibody, and positive immunostaining was not observed. These data indicate that TRPV4 is present in airway epithelial cells and in the vessel wall of medium-sized pulmonary arteries in the rat.

**DISCUSSION**

GSK1016790A is a recently described small-molecule agonist that potently activates TRPV4 channels in a variety of recombinant and naturally occurring biologic systems (22). The new findings in the present study are that the TRPV4 agonist GSK1016790A had potent vasodilator activity in systemic and pulmonary vascular beds of the rat when pulmonary vasoconstrictor tone was increased with U-46619. The present results also show that pulmonary vasodilator responses to the TRPV4 agonist are converted to a biphasic response with a potent pulmonary vasoconstrictor component when NOS is inhibited with l-NAME and that the systemic vasodilator response is enhanced, indicating that the responses to TRPV4 activation are modulated by NOS in a different manner in pulmonary and systemic vascular beds. Responses to GSK1016790A were attenuated by the TRPV4 antagonist GSK2193874, indicating that they are mediated by the activation of TRPV4 receptors.

The results of the present study show that intravenous injections of the TRPV4 agonist GSK101790A produce dose-related decreases in systemic arterial pressure, small decreases in pulmonary arterial pressure, and small increases in cardiac output, indicating that the TRPV4 agonist had marked vasodilator activity in the systemic vascular bed and modest pulmonary vasodilator activity under baseline conditions in the rat. In addition, when tone in the pulmonary vascular bed was increased to a high steady level with U-46619, the TRPV4 agonist had potent vasodilator activity in the pulmonary vascular bed. The results with GSK1016790A demonstrating that the TRPV4 agonist decreased systemic arterial pressure and systemic vascular resistance are consistent with results in a variety of species as well as results with the TRPV4 agonist 4α-phorbol-12,13-didecanoate in the rat (5, 22). The observation that the TRPV4 agonist had potent vasodilator activity in the pulmonary vascular bed when vasoconstrictor tone was increased to a high steady value has not been previously reported.

It has been shown that TRPV4 channels are expressed on vascular endothelial and smooth muscle cells in a variety of blood vessels and that activation of these channels induces vascular smooth muscle hyperpolarization, the release of NO from the endothelium, and vasodilation (2, 5, 9, 11, 12, 25). It has been hypothesized that TRPV4 channel activation represents the hyperpolarizing mechanism by which ACh induces endothelium-dependent vasodilation (7, 16, 17, 27). The vasorelaxant response to GSK1016790A in rat aortic rings was attenuated by endothelial denudation or l-NAME treatment.
and was not observed in aortic rings from endothelial NOS−/− mice, suggesting that vasorelaxant responses were endothelium and NO dependent (11). The effect of the NOS inhibitor L-NAME on hemodynamic responses to GSK1016790A was investigated in the rat, and decreases in systemic arterial pressure in response to intravenous injections of the TRPV4 agonist were greatly enhanced, whereas the pulmonary vasodilator response was converted to a biphasic response characterized by a large increase in pulmonary arterial pressure followed by a small secondary decrease in pulmonary arterial pressure. These data suggest that vasodilator responses to GSK1016790A in the systemic vascular bed are not mediated by NOS and NO release and are different than the results of a study (1) in isolated vessels. Moreover, the observation that GSK1016790A produced large increases in pulmonary arterial pressure when NO was inhibited with L-NAME may be interpreted to suggest that responses to the TRPV4 agonist are modulated by NOS and that TRPV4-mediated Ca2+ entry may promote vasoconstriction when NO formation is inhibited in the pulmonary vascular bed. The increase in pulmonary arterial pressure in response to GSK1016790A was attenuated by simultaneous coadministration of the TRPV4 agonist along with the Ca2+ entry antagonist isradipine. These results may suggest that TRPV4 receptor activation could result in increased Ca2+ entry through L-type Ca2+ channels in vascular smooth cells in small pulmonary arteries promoting vasoconstriction when NOS is inhibited with L-NAME. However, more data are needed to define this mechanism. The observation that L-NAME converted the pulmonary vasodilator response to GSK1016790A to a biphasic response with a potent pulmonary vasoconstrictor component may suggest that in pathophysiological conditions characterized by increased oxidative stress, which can inactivate NO, TRPV4 channel activation could increase hydrostatic pressure in the lung and enhance edema formation (24). It has been suggested that TRPV4 channel activation plays a role in pulmonary edema induced by elevated pulmonary venous pressure (2, 8, 26). It has been reported that TRPV4 receptor blockade prevented the increase in lung permeability and pulmonary edema associated with increased pulmonary venous pressure (18). The results of the present study suggest that TRPV4 activation can produce large increases in pulmonary arterial pressure when NO is inactivated and that this vasoconstrictor response can be blocked by the TRPV4 antagonist GSK2193874. The present results suggest that when endothelial function and NO formation are intact, TRPV4 activation could produce vasodilation in pulmonary and systemic vascular beds, reducing afterload on the right and left sides of the heart. Moreover, when NOS is inhibited and NO formation is impaired, the present results suggest that pulmonary vasoconstriction could occur and could increase afterload on the right ventricle and alter fluid balance in the lung.

In previous studies (22, 27), immunostaining, Western blot analysis, and RT-PCR demonstrated expression of TRPV4 in the vascular smooth muscle, endothelium, and airway epithelium from the rat lung. In addition, TRPV4 channels are expressed on sensory nerves and colocalize with calcitonin gene-related peptide and substance P (6, 10). The present results with immunostaining confirm the presence of TRPV4 in small- and medium-sized arteries and airways in the rat lung. A recent study (17) provided information on the interaction of TRPV4 channels with Ca2+ -activated K+ channels suggesting a mechanism by which TRPV4 activation induces endothelial and smooth muscle cell hyperpolarization and endothelium-dependent vasodilation. The observation that vasodilator responses are converted to vasoconstrictor responses in the pulmonary vascular bed when NOS is inhibited suggest that TRPV4 can play an important role in the regulation of pulmonary vasomotor tone and endothelial cell permeability and that TRPV4 may have an important role in the regulation of fluid balance in the lung.

In summary, the results of the present study show that a small-molecule potent activator of TRPV4 channels, GSK1016790A, can produce marked decreases in systemic vascular resistance and, under high pulmonary vascular tone conditions, marked decreases in pulmonary vascular resistance. Systemic vasodilator responses to the TRPV4 agonist were enhanced by the inhibition of NOS with L-NAME, and pulmonary vasodilator responses were converted to a biphasic response with a potent pulmonary vasoconstrictor component with large increases in pulmonary arterial pressure, which were attenuated by isradipine or the TRPV4 antagonist GSK2193874. These data suggest that pulmonary vascular responses to TRPV4 activation are modulated by NOS and the presence of NO and that systemic vasodilator responses do not require NOS, indicating that responses are modulated in a different manner in pulmonary and systemic vascular beds in the rat. These data may be interpreted to suggest that when NO formation or bioavailability is impaired, TRPV4 activation could increase pulmonary arterial pressure and enhance edema formation.

Fig. 7. Microscopic images at ×40 magnification showing TRPV4 immunostaining in a medium-sized pulmonary artery and airway from a rat lung. The images show positive TRPV4 immunoreactivity (white arrows, green) in the airway epithelium (A) and intima-media interface of the vessel lumen and smooth muscle (red; B) as well as nuclear staining (blue; C). Vessel lumen. Scale bar = 25 μm.
PULMONARY VASODILATOR RESPONSES TO THE TRPV4 AGONIST GSK1016790A

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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