Substance P–Induced Pulmonary Vasoreactivity in Isolated Perfused Guinea Pig Lung

William M. Selig, Kenneth E. Burhop, Joe G.N. Garcia, and Asrar B. Malik

We examined the effects of the neuropeptide substance P on pulmonary hemodynamic and transvascular fluid filtration in isolated Ringer’s-perfused and blood-enriched Ringer’s-perfused guinea pig lung and on albumin flux across bovine pulmonary artery endothelial monolayer. Mean pulmonary artery, left atrial, and capillary pressures were determined and used to calculate arterial and venous resistances, and lung weight was continuously monitored. Substance P (0.01–1.0 μM) caused marked increases in pulmonary arterial pressure, capillary pressure, venous resistance, and lung weight within 3–5 minutes after administration. These responses remained elevated above baseline at the end of the 30-minute experimental period in the Ringer’s-perfused lungs but not in the blood-enriched Ringer’s-perfused lungs. Substance P did not alter the capillary filtration coefficient in isolated lungs and transendothelial albumin permeability in the endothelial monolayer. Substance P resulted in an increase in venous effluent thromboxane B₂ concentrations in perfused lungs but had no effect on 6-keto-prostaglandin F₁α concentrations. Papaverine (0.27 mM) (a smooth-muscle relaxant) abolished the pulmonary microvascular response to substance P in Ringer’s-perfused lungs, and meclofenamate (0.15 mM) (a cyclooxygenase inhibitor) attenuated the pulmonary vasoconstriction and lung weight increase. Pyrilamine (1.0 μM) (a histamine-receptor antagonist) did not alter the responses to substance P. In conclusion, substance P does not affect pulmonary vascular permeability to water and protein. Substance P induces an intense pulmonary vasoconstriction (due to greater constriction of postcapillary vessels) and an elevation in pulmonary capillary pressure that increases net transvascular fluid filtration. The generation of cyclooxygenase metabolites contributes to substance P–induced pulmonary vasoactivity. (Circulation Research 1988;62:196–203)

Substance P (SP) is a biologically active undeca-peptide found in the central and peripheral nervous systems.¹ Pulmonary tissues innervated by neurons containing SP include arteries, large veins, bronchial blood vessels, respiratory smooth muscle, and respiratory epithelium of both the trachea and bronchi.²⁻⁴ Although primarily regarded as a neurotransmitter, SP release from peripheral sensory nerves may be an important neurotransmitter component in the inflammatory response.⁵⁻⁷

The physiological effects of SP on isolated pulmonary tissues include dose-dependent increases in tracheal smooth muscle and pulmonary artery smooth muscle tensions.⁸⁻⁹ Exogenous SP also induces an intense bronchoconstriction in several species examined.¹⁰⁻¹¹ The pulmonary hemodynamic response to SP appears to depend on basal tone of the pulmonary vasculature with the relaxed vasculature producing vasoconstriction and the preconstricted vasculature producing vasodilation.⁹⁻¹⁰ The systemic vascular effects of SP include hypotension associated with potent vasodilatation.¹¹ In the systemic circulation, SP also increases vascular permeability to protein.⁴

The purpose of the present study was to examine 1) the pulmonary microvascular response to SP (i.e., change in capillary pressure and vascular resistance) in isolated Ringer’s-perfused and blood-enriched Ringer’s-perfused guinea pig lungs, 2) the effect of SP on pulmonary vascular permeability as measured by the capillary filtration coefficient in the isolated perfused lung and radiolabeled albumin permeability in the pulmonary endothelial monolayer, and 3) the postulated roles of cyclooxygenase metabolites and histamine in pulmonary microvascular responses to SP.

Materials and Methods

Isolated Perfused Guinea Pig Lung

Guinea pigs (Buckberg Laboratories, Tomkins Cove, New York) weighing 430 ± 39 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.; Abbott, Chicago, Illinois), and tracheotomized. Following a thoracotomy, intracardiac heparin (700 U/kg, Invenex, Chagrin Falls, Ohio) was administered, and the animals were exsanguinated. The lungs and heart were removed together and suspended from one end of a counterweighted beam balance to monitor lung weight. The pulmonary artery and left atrium were cannulated. Perfusion was begun at a low flow rate within 5 minutes of pneumothorax using a peristaltic roller pump (model 1215, Harvard Apparatus, Millis, Massachusetts). Airway pressure was maintained at 1 cm H₂O with 95% O₂, 5% CO₂, and venous outflow pressure was set at 3 cm H₂O so that all lungs remained in the Zone III condition. Lungs were covered with Saran wrap to prevent evaporative fluid loss. Recircu-

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ml/min was begun when the venous effluent was clear of blood.

The perfusion system used in the present study has been previously described. Continuous pulmonary wet-weight recordings were made on a three-channel Gould recorder (model 2200S, Cleveland, Ohio), calibrated so that a 0.5-g weight change resulted in a 6-cm recorder pen deflection. Pulmonary arterial and left atrial pressures were continuously monitored with transducers (Statham P50 and Gould P23ID) connected to catheters (PE-90) placed in the pulmonary artery and left atrium. All monitored variables were stable, and lungs without intervention remained isogravimetric for up to 3 hours. All studies involving substance P challenge were confined to 1 hour or less.

The perfusate consisted of either a phosphate-buffered Ringer's solution containing 0.5% bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, Missouri) or blood diluted with Ringer's solution. The phosphate-buffered Ringer's solution consisted of (in mM): NaCl 137, CaCl2 1.8, MgCl2 1.05, KCl 2.68, NaHCO3 0.06, NaH2PO4 0.130, Na2HPO4 0.869, and dextrose 5.55. Blood was obtained from heparinized (700 U/kg) donor guinea pigs by direct cardiac puncture. The heparinized blood was mixed with heparinized Ringer's solution in the following dilution (2-3-second), simultaneous occlusion of both arterial inflow and venous outflow during which arterial and venous outflow pressures were simultaneously elevated to 3 cm H2O for 5 minutes. Pulmonary capillary pressures were estimated immediately before the increase in Pp and at the end of the 5-minute elevation in Pp to obtain the change in capillary pressure. The resulting increase in lung weight corresponded to a two-compartment model: 1) a rapid component attributed to vascular filling and 2) a slower component representing an increase in the interstitial volume attributed to transvascular fluid filtration. The Kf was calculated according to the method of Drake. The rate of lung weight gain was calculated for each minute following the rise in Pp, and was expressed as a semilogarithmic function over time. The slow component of weight gain (corresponding to the increase in interstitial volume) was extrapolated to time 0 to obtain an estimate of the fluid filtration rate, which was divided by the change in Pp to obtain Kf. At the end of each experiment, the lungs were dissected free of nonpulmonary tissue and dried to a constant weight at 50°C. Kf was expressed in ml/min·cm H2O·g dry lung wt. Lungs were discarded if they did not reach an isogravimetric state following the initiation of perfusion.

Pulmonary capillary pressure and vascular resistance. Pulmonary capillary pressure (Pcp) was estimated by either the double-occlusion technique or the isogravimetric method. Previous estimates using venous and double-occlusion techniques agree with Pcp values obtained using the gravimetric technique used to measure mean filtration pressure. The double-occlusion technique consisted of a brief (2-3-second), simultaneous occlusion of both arterial inflow and venous outflow during which arterial pressure (Ppa) decreased and venous pressure (Pv) increased to an equilibrium pressure that approximated Pcp. The pulmonary vascular isogravimetric Pcp was also estimated by the procedure developed by Pappenheimer and Soto-Rivera and modified by Gaar. Following establishment of an isogravimetric state, a perfusate flow reduction caused a decrease in arterial pressure and a concomitant loss in lung weight. The venous outflow pressure was simultaneously elevated until the lung neither lost nor gained weight. At least four isogravimetric states were obtained at different flow rates to determine each isogravimetric Pcp, Ppa and Pp were then plotted as a linear function of perfusate flow and fit by least-squares regression. The intersection of the Pp and P lines near the pressure axis represented the isogravimetric Pcp (determined by the double-occlusion or isogravimetric methods) was used to partition resistance into upstream, or arterial, (Rpa) and downstream, or venous, (Rpv) segments from the following equations, where Q equals flow:

\[
R_p = \frac{P_{pa} - P_{cp}}{Q}
\]

and

\[
R_v = \frac{P_{cp} - P_v}{Q}
\]

Capillary filtration coefficient. The capillary filtration coefficient (Kf) was measured at specific intervals in Ringer's-perfused lungs treated with the smooth-muscle relaxant papaverine (0.27 mM) to minimize hydrostatic pressure changes produced by SP administration or in blood-enriched Ringer's-perfused lungs. Following an isogravimetric period, the outflow pressure was rapidly elevated by 3 cm H2O for 5 minutes. Pulmonary capillary pressures were estimated immediately before the increase in Pp and at the end of the 5-minute elevation in Pp to obtain the change in capillary pressure. The resulting increase in lung weight corresponded to a two-compartment model: 1) a rapid component attributed to vascular filling and 2) a slower component representing an increase in the interstitial volume attributed to transvascular fluid filtration. The Kf was calculated according to the method of Drake. The rate of lung weight gain was calculated for each minute following the rise in Pp, and was expressed as a semilogarithmic function over time. The slow component of weight gain (corresponding to the increase in interstitial volume) was extrapolated to time 0 to obtain an estimate of the fluid filtration rate, which was divided by the change in Pp to obtain Kf. At the end of each experiment, the lungs were dissected free of nonpulmonary tissue and dried to a constant weight at 50°C. Kf was expressed in ml/min·cm H2O·g dry lung wt. Lungs were discarded if they did not reach an isogravimetric state following the initiation of perfusion.

Radioimmunoassay of arachidonic acid metabolites. Venous effluent samples were collected from the left atrial cannula for analysis of the thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-keto-PGF1α) (stable degradation products of thromboxane A2 and prostacyclin, respectively) by the double-antibody radioimmunoassay technique. The specifics of the radioimmunoassay have been previously described. All samples were stored in polypropylene tubes containing indomethacin (0.17 mM) at −70°C. Organic extraction to remove possible interfering proteins was not required since plasma standards were included in this assay. The cross-reactivities of the 6-keto-PGF1α antiserum were 3.3% prostaglandin F1α (PGF1α), 0.1% prostaglandin D1 (PGD1), 0.24% prostaglandin E2 (PGE2), 0.27% prostaglandin F2α (PGF2α), and <0.01% TXB2, arachidonic acid, or linoleic acid. The cross-reactivities of the TXB2 antiserum were 0.06% PGD2, and <0.01% for PGE2, PGE2α, PGF2α, 6-keto-PGF1α, arachidonic acid, or linoleic acid. Linear working ranges for both antisera were established between 5 and 120 pg.
**Endothelial Cell Monolayer**

Polycarbonate micropore filters (13-mm diameter, 0.8-μm pore size, Nuclepore, Pleasanton, California) were gelatinized according to the method of Postlewaite and as modified by Taylor. The micropore filter was glued onto the base of a plastic cylinder (11-mm i.d., Adaps, Dedham, Massachusetts) and sterilized by ultraviolet light for 24 hours. The bovine pulmonary artery endothelial cell line (CCL-209) of Del Vecchio and Smith was obtained at 16 passages from the American Culture Collection (Rockville, Maryland). At 18 passages, 0.5 ml of cells (4 × 10^5 cells) suspended in culture medium were seeded onto the gelatinized filter. The system consisted of two compartments communicating only via the microporous polycarbonate membrane, on one side of which the endothelial monolayer was grown. The endothelial cell monolayer was then assembled for the study of transendothelial albumin transport under a condition devoid of hydrostatic pressure because the upper chamber was floated in the larger lower chamber so that the fluid levels in both chambers were the same. The upper compartment (volume 700 μl) contained bovine serum albumin (4 g%) and a tracer amount of \[^{125}\text{I}\] albumin mixed in Dulbecco’s Modified Eagle Medium (DMEM) (Gibco Lab, Grand Island, New York), and the lower compartment (volume 25 ml) contained DMEM with albumin (4 g%). The lower compartment was stirred continuously to mix the contents, and the entire system was kept in a thermostatically controlled water bath at 37° C. The albumin clearance was measured for 60 minutes (preintervention) and for 60 minutes (postintervention) following administration of a test substance (total study period, 90 minutes). The albumin clearance was determined by taking two 25-μl samples from the upper compartment at the beginning of each experiment, serial 400-μl samples from the bottom compartment every 5 minutes for the first 30 minutes, and then 400-μl samples at 10-minute intervals for an additional 60 minutes. Radioactive counts of the samples were measured using a gamma scintillation counter (model ND60, Nuclear Data, Shaumberg, Illinois). To minimize errors due to diffusion of free radioactive iodine, labeled albumin was diazylated continuously and was not used unless the free iodine was less than 1.0%.

**Data analysis.** The clearance rate of \[^{125}\text{I}\] albumin (Cl\(_{\text{ab}}\)) for each monolayer was obtained for the first 30-minute period (baseline or preintervention) and for the next 60-minute period (postintervention). Cl\(_{\text{ab}}\) was calculated as

\[
\text{Cl}_{\text{ab}} = \frac{V_a[A]}{[L]}
\]

where \(V_a\) is the lower compartment volume at each time point (corrected for each sampling volume change), \([A]\) is the lower compartment tracer (albumin) concentration at each time point, and \([L]\) is the upper compartment tracer (albumin) concentration at each time point. The clearance rate (μl/min) was calculated by fitting the measured clearance rate for each portion of each respective experiment to two straight lines (i.e., preintervention and postintervention) by least-squares linear regression.

**Experimental protocols.** SP (Peninsula Laboratories, Belmont, California) was stored at ~20° C in a dessicator and dissolved in 0.9% saline at the time of the experiment. Different concentrations were administered by mixing SP into the perfusion reservoirs of isolated Ringer’s-perfused or blood-enriched Ringer’s-perfused lungs after establishing baseline conditions (final recirculating reservoir concentrations 0.01–1.0 μM). In another experimental group, double-occlusion capillary pressures were simultaneously compared with isogravimetric capillary pressures after SP administration (0.1 μM) in the same Ringer’s-perfused guinea pig lung (n = 5).

In a separate group of isolated Ringer’s-perfused lungs, one of the following agents was added to the perfusate reservoir and recirculated for 20 minutes prior to SP (0.1 μM) administration: papaverine, a smooth-muscle relaxant (0.27 mM, Sigma Chemical, St. Louis, Missouri, n = 6); sodium meclofenamate, a cyclooxygenase inhibitor (0.15 mM, Warner-Lambert, Ann Arbor, Michigan, n = 6); or pyrilamine, a histamine-receptor antagonist (1.0 μM, Sigma Chemical, n = 4). Vehicle or drug controls did not alter pulmonary hemodynamics, lung wet weight, or the capillary filtration coefficient in isolated Ringer’s-perfused or blood-enriched Ringer’s-perfused lungs for up to 3 hours.

In the endothelial monolayer system, changes in albumin clearance were measured in the presence of high dose SP (1.0 mM, n = 6) and oleic acid, a positive control (4.0 mM, n = 7).

**Statistical analysis.** Data from the perfused lung and monolayer experiments were expressed as mean ± SEM. Statistical analysis in the perfusion experiments was performed using repeated measures analysis of variance followed by multiple comparisons testing using the Bonferroni t test. Significant changes in the clearance rate between preintervention versus postintervention periods in the monolayer experiments were calculated using the unpaired Student’s t test. Statistical significance was accepted at \(p < 0.05\).

**Results**

A representative tracing of the response to SP (0.1 μM) in the Ringer’s-perfused lung is shown in Figure 1. Three different recirculating concentrations of SP (0.01, 0.1, and 1.0 μM) produced an increase in pulmonary arterial pressure (\(P_a\)) in the isolated Ringer’s-perfused lung (Figure 2). At the lower two concentrations of SP (0.01 and 0.1 μM), peak increases in \(P_a\) over baseline (52 ± 11% and 80 ± 6% above baseline, respectively) were observed at 5 minutes after SP administration. As shown in Figure 2, the increase in \(P_a\) in the 0.1- and 1.0-μM SP–treated groups was sustained during the 30-minute perfusion. The SP-induced changes in \(P_a\) were abolished in the presence of papaverine (0.27 mM) while meclofenam-
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Weight (g)  

Figure 1. Representative tracing from an isolated Ringer's-perfused lung experiment showing the effects of substance P (0.1 μM) on pulmonary arterial pressure (P<sub>a</sub>), capillary pressure (P<sub>cap</sub>), venous pressure (P<sub>v</sub>), and lung weight. P<sub>cap</sub> measurement was recorded at a faster chart recorder speed for approximately 5 seconds during the 2-3-second occlusion period.

mate (0.15 mM) attenuated the response (Figure 3). Pyrilamine (1.0 μM) did not affect the SP-induced increase in P<sub>cap</sub> (Figure 3). As shown in Table 1, SP (0.1 μM) produced an increase in P<sub>cap</sub> in blood-enriched Ringer's-perfused lungs (peak increase of 115 ± 10% above baseline), which was similar to that seen in Ringer's-perfused lungs. In contrast to the Ringer's-perfused lungs, the SP-induced pressor response was not sustained in blood-enriched Ringer's-perfused lungs (Table 1).

Figure 2 illustrates the effects of SP on pulmonary capillary pressure (P<sub>cap</sub>) estimated using the double-occlusion method. P<sub>cap</sub> increased in all instances following SP administration. The changes in P<sub>cap</sub> after SP administration (0.1 μM) measured using the double-occlusion method agreed with measurements obtained using the isogravimetric method (Table 2). Papaverine and meclofenamate significantly (p<0.05) attenuated the response (Figure 3). Pyrilamine (1.0 μM) did not affect the SP-induced increase in P<sub>cap</sub> (Figure 3). As shown in Table 1, SP (0.1 μM) produced an increase in P<sub>cap</sub> in blood-enriched Ringer's-perfused lungs (peak increase of 115 ± 10% above baseline), which was similar to that seen in Ringer's-perfused lungs. In contrast to the Ringer's-perfused lungs, the SP-induced pressor response was not sustained in blood-enriched Ringer's-perfused lungs (Table 1).

Figure 3. Effect of substance P (SP) (0.1 μM) on pulmonary arterial (P<sub>a</sub>, top) and capillary pressure (P<sub>cap</sub>, bottom) in isolated Ringer's-perfused lungs pretreated with meclofenamate (Meclo, 0.15 mM, n = 6), papaverine (Papav, 0.27 mM, n = 6), or pyrilamine (Pyril, 1.0 μM, n = 4). SP was administered following baseline (time 0) measurements. Values are mean ± SEM. *p<0.05 different from baseline value.
Table 1. Effect of Substance P on Pulmonary Hemodynamics and Transvascular Fluid Filtration in Blood-Enriched Ringer’s-Perfused Guinea Pig Lung

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (minutes)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>Arterial pressure (cm H₂O)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.8 ± 0.4</td>
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<tr>
<td>Capillary pressure (cm H₂O)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>Arterial resistance (cm H₂O/ml/min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Venous resistance (cm H₂O/ml/min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Change in lung wet weight from baseline (g)</td>
<td>0</td>
</tr>
<tr>
<td>Capillary filtration coefficient (ml/min-cm H₂O g dry lung wt)</td>
<td>0.0068 ± 0.0013</td>
</tr>
</tbody>
</table>

Substance P (0.1 μM, n = 5) was administered immediately after baseline measurements. Mean ± SEM. *p < 0.05 different from baseline value.

reduced SP-induced increases in Pₑₑₑ, while pyrilamine had no effect (Figure 3). SP (0.1 μM) also produced an increase in Pₑₑₑ in blood-enriched Ringer’s-perfused lungs, although the response was not sustained (Table 1).

The effects of SP on pulmonary arterial (Rₐ) and venous (Rᵥ) resistances in the guinea pig lung are shown in Table 3. Rₐ was not significantly altered after SP at any concentration, while Rᵥ was increased markedly following SP at all concentrations (Table 3). Both papaverine and meclofenamate attenuated the SP-induced increase in Rᵥ, while pyrilamine had no effect (Table 4). SP (0.1 μM) produced a marked increase in Rᵥ but not in Rₐ in blood-enriched Ringer’s-perfused lungs, although the response was not sustained (Table 1).

The effects of SP administration on lung wet weight in the isolated perfused lung are shown in Figure 4. Lung weight at the beginning of perfusion averaged 3.39 ± 0.14 g (n = 10). SP (0.1 μM) induced a marked increase in pulmonary wet weight (peak increase approximately 50% above baseline), which was abolished by papaverine and attenuated by meclofenamate but was not affected by pyrilamine (Figure 4). In contrast, the SP-induced weight change in blood-enriched Ringer’s-perfused lungs was not sustained (Table 1).

SP (0.1 μM) administration caused a transient increase in venous effluent TXB₂ concentrations in the isolated Ringer’s-perfused guinea pig lung, and meclofenamate abolished this response (Figure 5). Concentrations of 6-keto-PGF₇α after SP administration (0.1 μM) were not significantly different from baseline (Figure 5).

SP (0.1 μM) did not alter the pulmonary capillary filtration coefficient (Kᵥ) measured in Ringer’s-perfused lungs (n = 6). Baseline Kᵥ values averaged 0.024 ± 0.002 ml/(min-cm H₂O g dry lung wt). Mean Kᵥ values measured at 5, 10, 20, and 30 minutes after SP administration were 0.019 ± 0.004, 0.021 ± 0.005, 0.019 ± 0.005, and 0.020 ± 0.004 ml/(min-cm H₂O g dry lung wt), respectively. SP (0.1 μM) also did not alter the Kᵥ in blood-enriched Ringer’s-perfused lungs (Table 1).

The effect of SP on [¹²⁵I]albumin permeability across the bovine pulmonary artery endothelial monolayer system is shown in Table 5. The endothelial cells restricted albumin when compared with the gelatinized filter on which the cells were grown. SP did not increase transendothelial [¹²⁵I]albumin clearance at any concentration. The free fatty acid oleic acid (a positive control) markedly increased albumin clearance in this monolayer system.

Discussion

The present experiments show that SP caused a marked pulmonary vasoconstriction and an elevation...
in pulmonary capillary pressure in both the isolated Ringer's-perfused and blood-enriched Ringer's-perfused guinea pig lung. Studies using isolated rabbit9 and guinea pig30 pulmonary artery segments show that SP produces a dose-dependent increase in pulmonary artery tension. The SP-induced response in isolated pulmonary arterial segments appears to depend on the baseline tension of the preparation.9 Both Worthen13 and Archer14 demonstrated that SP-induced pulmonary vasodilation occurs when the pulmonary vasculature is in a preconstricted state. In anesthetized dogs, SP injection increased pulmonary arterial pressure and caused pulmonary vasodilatation.12 Previous studies on the pulmonary vascular effects of SP examined pulmonary arterial strip tension9-30 or total pulmonary vascular resistance13 but have not compared the responses between segments of the pulmonary vasculature. In the present study, an increase in resistance of downstream vessels was observed (probably pulmonary veins) but not a change in pulmonary arterial resistance after SP administration. The absence of pulmonary arterial constriction may reflect differences in baseline pulmonary vascular tone in our preparation or the reversal of pulmonary arterial constriction induced by upstream blood pooling secondary to intense pulmonary venoconstriction. The present data indicate that SP is a potent pulmonary vasodilator and that this effect appears to be confined to pulmonary veins.

We used both the double-occlusion17,18 and isogravimetric19,21 method to partition pulmonary vascular resistances into the precapillary (upstream) and post-capillary (downstream) segments. Both techniques were used since assumptions about the greater compliance of the middle vascular segment were required for the occlusion method but not the isogravimetric method.17,18,21 The isogravimetric capillary pressure method partitions resistance upstream and downstream from the filtering vessels18,21 and does not require assumptions about differential compliance of pulmonary vascular segments. With both methods, the increase in vascular resistance with SP occurred primarily in the downstream vessels. The finding that lung weight increased after SP administration also supports the notion that pulmonary veins are the primary sites of constriction.

The factors that induce the release of SP in vivo are unknown. SP-immunoreactive nerve fibers are found in close proximity to pulmonary vessels,31,32 and it is conceivable that activation of these fibers and the subsequent release of SP induces an increase in pulmonary vasmotor tone.

The pulmonary microvascular response to SP was not sustained at the lowest concentration of SP (0.01 μM) in Ringer's-perfused lungs or blood-enriched Ringer's-perfused lungs. The decrease in the response may reflect the capacity of pulmonary endothelial and plasma enzymes to catabolize SP. Previous studies in anesthetized rats indicate that SP undergoes partial inactivation in the pulmonary vascular bed31 by aminopeptidases32 and angiotensin converting enzyme.33 Studies using human plasma also indicate that SP is rapidly inactivated to less biologically active metabolites.34 The rapid metabolism of SP by plasma

<table>
<thead>
<tr>
<th>Substance P concentration (μM)</th>
<th>0.01</th>
<th>0.1</th>
<th>1.0</th>
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</thead>
<tbody>
<tr>
<td>Time (minutes)</td>
<td>Ra</td>
<td>Rv</td>
<td>Ra</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.09±0.01</td>
<td>0.07±0.02</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.07±0.01</td>
<td>0.21±0.05*</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.07±0.01</td>
<td>0.22±0.06*</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>10</td>
<td>0.06±0.02</td>
<td>0.20±0.05*</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>20</td>
<td>0.06±0.01</td>
<td>0.16±0.06*</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.06±0.01</td>
<td>0.14±0.04*</td>
<td>0.04±0.01*</td>
</tr>
</tbody>
</table>

Rp, pulmonary arterial resistance (cm H2O/[ml/min]); Rv, pulmonary venous resistance (cm H2O/[ml/min]); SP, substance P (0.1 M); papav, papaverine (0.27 mM); meclo, meclofenamate (0.15 mM); pyril, pyrilamine (1.0 M); mean ± SEM. Substance P was administered immediately after baseline measurements. *p<0.05 different from baseline value. †Different (p<0.05) from SP alone at the corresponding time.
components may partially explain the transient response to SP in blood-enriched Ringer's-perfused lungs as compared with the Ringer's-albumin-perfused lungs. The mechanism of SP-induced pulmonary venoconstriction in the isolated perfused guinea pig lung is not clear. Since papaverine abolished the SP-induced pulmonary venoconstriction, the response is probably the result of pulmonary vascular smooth muscle contraction. The smooth-muscle relaxant action of papaverine may be due to a calcium channel blocking effect and to elevation of intracellular cAMP, suggesting that SP may act through these messenger systems. Meclofenamate attenuated the pulmonary vascular response to SP administration in Ringer's-perfused lungs indicating the involvement of cyclooxygenase metabolites in the response. Studies in isolated rabbit pulmonary artery and the anesthetized rabbit have shown that SP-induced pulmonary vascular smooth-muscle contraction and pulmonary vasoconstriction were attenuated in the presence of cyclooxygenase or thromboxane synthetase inhibitors. The present study also indicates that thromboxane generation may contribute to SP-induced pulmonary venoconstriction. Thromboxane is a likely mediator since it is known to cause venoconstriction in the pulmonary vasculature. SP-induced thromboxane generation occurs from some lung parenchymal cell since it was observed in lungs perfused with Ringer's-albumin solution. Substance P is known to induce thromboxane generation from guinea pig macrophages.

Histamine has a described pulmonary venoconstrictor effect; moreover, SP causes histamine release from rat peritoneal and intestinal mucosal mast cells. Therefore, we tested the role of histamine in the SP-induced pulmonary vasoconstriction. The histamine-receptor antagonist pyrilamine in the present study did not alter the pulmonary microvascular response to SP, indicating that histamine is not responsible for the action of SP. Histamine receptor antagonists were also ineffective in reducing SP-induced contraction of rabbit tracheal smooth muscle segments and pulmonary artery segments and of guinea pig lung strips. SP did not alter the capillary filtration coefficient in the isolated perfused guinea pig lung and transendothelial albumin permeability in the endothelial monolayer. Although SP caused increases in lung wet weight, these changes are mediated by the increase in pulmonary capillary hydrostatic pressure secondary to SP-induced pulmonary venoconstriction. In contrast with the present study, intravenous SP (0.003 μM/kg) increased the flux of Evan's blue dye across the pulmonary vessels, airways, and splanchnic vessels of anesthetized guinea pigs. SP-induced vasodilation and plasma extravasation were also seen in the rat hind paw. The Evan's blue dye extravasation may be the result of SP-induced pulmonary capillary hypertension and increased vascular surface area rather than an increase in vascular permeability.

<table>
<thead>
<tr>
<th>Filter (n = 6)</th>
<th>1.415 ± 0.085</th>
<th>1.208 ± 0.030</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium (n = 8)</td>
<td>0.336 ± 0.050</td>
<td>0.285 ± 0.071</td>
</tr>
<tr>
<td>Endothelium + substance P (n = 6)</td>
<td>0.359 ± 0.026</td>
<td>0.362 ± 0.027</td>
</tr>
<tr>
<td>Endothelium + oleic acid (n = 7)</td>
<td>0.258 ± 0.019</td>
<td>0.624 ± 0.082*</td>
</tr>
</tbody>
</table>

*p < 0.05 different from preintervention value.
In summary, SP administration in the isolated Ringer's-perfused and blood-enriched Ringer's-perfused guinea pig lung resulted in marked pulmonary vasoconstriction, which was likely due to contribution of the vessels downstream from the fluid filtration site. SP-induced vasoconstriction also produced an increase in lung wet weight as a result of the rise in pulmonary capillary hydrostatic pressure. There was no direct permeability-increasing effect of SP in the pulmonary microcirculation. Pulmonary vasoconstriction was abolished by papaverine and attenuated by cyclooxygenase inhibition, indicating that the response is the result of pulmonary vascular smooth muscle contraction mediated in part by the generation of cyclooxygenase-derived arachidonic acid metabolites.

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Key Words • substance P • guinea pig lungs • papaverine • cyclooxygenase metabolites • histamine • pulmonary vasoconstriction • endothelial monolayer • vascular permeability • capillary hydrostatic pressure
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