

Garlic prevents hypoxic pulmonary hypertension in rats

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Fallon, Michael B., Gary A. Abrams, Tarek T. Abdel-Razek, Jun Dai, Shi-Juan Chen, Yiu-Fai Chen, Bao Luo, Suzanne Oparil, and David D. Ku. Garlic prevents hypoxic pulmonary hypertension in rats. *Am. J. Physiol. Cell. Mol. Physiol.* 19: L283–L287, 1998.—Hypoxic pulmonary vasoconstriction underlies the development of high-altitude pulmonary edema. Anecdotal observations suggest a beneficial effect of garlic in preventing high-altitude symptoms. To determine whether garlic influences pulmonary vasoconstriction, we assessed the effect of garlic on pulmonary pressures in rats subjected to alveolar hypoxia and on vasoconstriction in isolated pulmonary arterial rings. Garlic gavage (100 mg/kg body wt) for 5 days resulted in complete inhibition of acute hypoxic pulmonary vasoconstriction compared with the control group. No difference in mean arterial pressure or heart rate response to hypoxia was seen between the groups. Garlic solution resulted in a significant dose-dependent vasorelaxation in both endothelium-intact and mechanically endothelium-disrupted pulmonary arterial rings. The administration of *N*^G-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor) inhibited the vasodilatory effect of garlic by 80%. These studies document that garlic blocks hypoxic pulmonary hypertension *in vivo* and demonstrate a combination of endothelium-dependent and -independent mechanisms for the effect in pulmonary arterial rings.

nitric oxide synthase; *Allium sativum*; vascular rings

ALLIUM SATIVUM (garlic) has been employed as an herbal medicinal agent for thousands of years (11). Among the beneficial effects attributed to garlic is the ability to relax vascular smooth muscle (16). In the early 19th century during Argentina's fight for independence, San Martin observed that administration of garlic to his soldiers while they crossed the Andes countered the effects of high altitude (11). One explanation for a beneficial effect of garlic ingestion in these soldiers might have been amelioration of hypoxic pulmonary vasoconstriction. Recent experimental studies have demonstrated several effects of garlic, including activation of endothelial nitric oxide synthase (6) and smooth muscle cell membrane hyperpolarization (18), which could decrease pulmonary vascular tone. Together, these observations suggest that garlic administration might block the development of hypoxic pulmonary vasoconstriction by inhibiting pulmonary vascular smooth muscle contraction.

The aim of the present study was to evaluate whether garlic administration alters the development of hypoxic pulmonary vasoconstriction and to assess the potential mechanisms for the effect on pulmonary vascular tone. Here, we determine the effects of enteral garlic administration on the development of pulmonary vasoconstriction in a rat model of hypoxic pulmonary hypertension

and investigate the mechanisms through which garlic influences the pulmonary arterial system in isolated pulmonary arterial segments.

METHODS

Animals. Male Sprague-Dawley rats weighing 280–320 g were obtained from Charles River Breeding Laboratories (Wilmington, MA). For acute hypoxic studies, five garlic-treated and nine control animals were used. For pulmonary arterial ring studies, a minimum of 10 rings obtained from 5 separate animals were studied for each experimental group. The study was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham and conforms to National Institutes of Health guidelines on the care and use of laboratory animals.

Garlic administration. Dehydrated garlic powder was obtained from Pure-Gar (Tacoma, WA). For gavage administration, a garlic solution was prepared daily by mixing dehydrated garlic powder with 5% gum arabic in double-distilled water. The mixture was vortexed for 5 min and administered by gavage (100 mg garlic powder/kg body wt) daily for 5 days. Control animals were given 5% gum arabic solution alone for 5 days. For pulmonary arterial ring studies, dehydrated garlic powder was mixed 1:9, wt/vol, with double-distilled water, followed by vortexing for 5 min and centrifugation at 8,000 *g* for 5 min. The supernatant was then diluted to appropriate concentrations for vessel reactivity testing.

Vascular catheterization. On the fourth gavage day, femoral arterial and venous cannulas and pulmonary arterial cannulas were inserted under pentobarbital sodium anesthesia in the rats through the right jugular vein with a closed-chest technique as previously described (5, 15). A small incision was made in the proximal right external jugular vein through which an introducer and a Silastic catheter (PE-10) were passed. The catheter was filled with a heparin-saline solution, attached by a 25-gauge blunted needle to a pressure transducer (model CP-01, Century Technology, Inglewood, CA) coupled to a polygraph (model 7, Grass Instruments, Quincy, MA), and advanced through the introducer into the pulmonary artery. Catheter position was identified by the pressure tracing, and the introducer was removed after the proper position was confirmed. The catheter was secured and then connected to polyethylene tubing (PE-10 fused to PE-20), which was exteriorized at the back of the neck.

Acute hypoxic studies. Acute hypoxic studies were accomplished as previously described (5). Thirty-six hours after catheterization, mean systemic arterial pressure (MSAP), mean pulmonary arterial pressure (MPAP), and heart rate (HR) were recorded through the femoral and pulmonary arterial catheters. After stable MSAP and MPAP recordings were obtained, the conscious unrestrained rats were exposed to hypoxia (10% O₂) for 90 min in a 330-liter Plexiglas glove box (Manostat, Brooklyn, NY) that was gassed by adding N₂ (Southern Welding, Birmingham, AL) to the chamber intermittently from a liquid N₂ reservoir. The gas outflow of N₂ was controlled by a solenoid valve activated by the recorder output of an S3-A O₂ analyzer (Applied Electrochemistry,

Sunnyvale, CA) through a control circuit (model 371-K, LFE, Clinton, MA), which ensures uniform and reproducible lowering of O₂ concentrations within the chamber. A baralyme (Allied Health Care Products, St. Louis, MO) CO₂ scrubber kept the CO₂ concentration at <0.2%. Relative humidity within the chamber was kept at <70% with anhydrous CaSO₄. Boric acid was used to keep NH₃ levels within the chamber at a minimum. A double-doored entry port allowed the animals to be placed into the chamber without opening it to room air and without producing measurable alterations in the O₂ concentration of the internal atmosphere.

Pulmonary arterial ring studies. On the day of the experiment, the rats were anesthetized with pentobarbital sodium (60 mg/kg ip), the thoracic cavity was opened, and both the left and right main lobes of lung were quickly excised. The intralobar pulmonary arteries, ~1.5 cm in length, were carefully dissected from the surrounding connecting tissues under a stereomicroscope. The arteries were cut into rings (2–3 mm long), with the outside diameter ranging from 0.5 to 1.2 mm. Each ring was then mounted, as previously described (8), with two triangular-shaped 30-gauge stainless steel needles in jacketed tissue chambers containing Krebs-Henseleit solution (in mM: 118 NaCl, 4.6 KCl, 27.2 NaHCO₃, 1.2 MgSO₄, 1.2 KH₂PO₄, 1.75 CaCl₂, 0.03 Na₂EDTA, and 11.1 glucose) at 37°C gassed with 95% O₂-5% CO₂. The upper needle of each arterial ring was attached with a silk suture to a force-displacement transducer (Grass FT 0.03C), and changes in isometric force were recorded on a Grass polygraph (model 7C).

To assess the role of the endothelium in the arterial ring response to garlic, the endothelium was denuded from a subset of isolated pulmonary arterial segments by mechanical disruption with a wooden applicator before vascular reactivity studies. Effectiveness of the endothelial disruption was assessed in each vessel by documenting a complete loss of relaxation in response to acetylcholine (1 μM) challenge.

All vessels were passively stretched to generate a resting tension of 2.0 g for isometric contraction recording. After 40 min of equilibration, the rings were exposed to maximum depolarizing 70 mM KCl. When contractile responses plateaued, ranging from 0.4 to 0.7 g of active tension, the rings were rinsed with Krebs-Henseleit solution and allowed to equilibrate for an additional 40 min in the presence of 5 μM indomethacin before the start of the experiment. At appropriate times, submaximal tone was elicited with 0.1–1 μM phenylephrine, and various pharmacological agents were added to the bath. In all cases, the effects of only one dose-response curve of acetylcholine or aqueous garlic extract solution was determined in each pulmonary segment, except for the cases when repeat studies of acetylcholine or garlic solution were indicated after pretreatment with the nitric oxide synthase inhibitor *N*^G-nitro-L-arginine methyl ester (L-NAME). Data are expressed as a percentage of relaxation or a percentage of decrease in phenylephrine-induced constrictive tone.

Reagents. Acetylcholine chloride, phenylephrine, and L-NAME were purchased from Sigma (St. Louis, MO), and all other reagents were purchased from Fisher Scientific (Norcross, GA). Acetylcholine was prepared in sodium acetate buffer (pH 4.0) and stored at 4°C to ensure stability. All other solutions were prepared immediately before use.

Statistics. Data were analyzed with Student's *t*-test, ANOVA, and multiple comparisons between groups with Bonferroni correction as appropriate. All measurements are expressed as means ± SE. Statistical significance was designated as *P* < 0.05.

RESULTS

Effects of garlic gavage on the vascular response to acute normobaric hypoxia. To determine whether chronic garlic ingestion alters the pulmonary vascular responsiveness to normobaric hypoxia, we subjected control and garlic-gavage animals to a 90-min exposure of 10% O₂ after 5 days of garlic gavage. Figure 1 summarizes the responses of MPAP, MSAP, and HR to acute hypoxic exposure in control and garlic-gavage animals. In control rats, acute hypoxic exposure was associated with a significant increase in MPAP of 8–10 mmHg from baseline, beginning within 2 min of exposure and persisting for the entire 90-min study (Fig. 1A). In contrast, animals treated with garlic gavage for 5 days before the hypoxic challenge had no significant increase in MPAP during acute hypoxic exposure and were significantly different from control animals. MSAP fell similarly by ~10 mmHg over 90 min in both the control and garlic-treated animals (Fig. 1B). HR increased

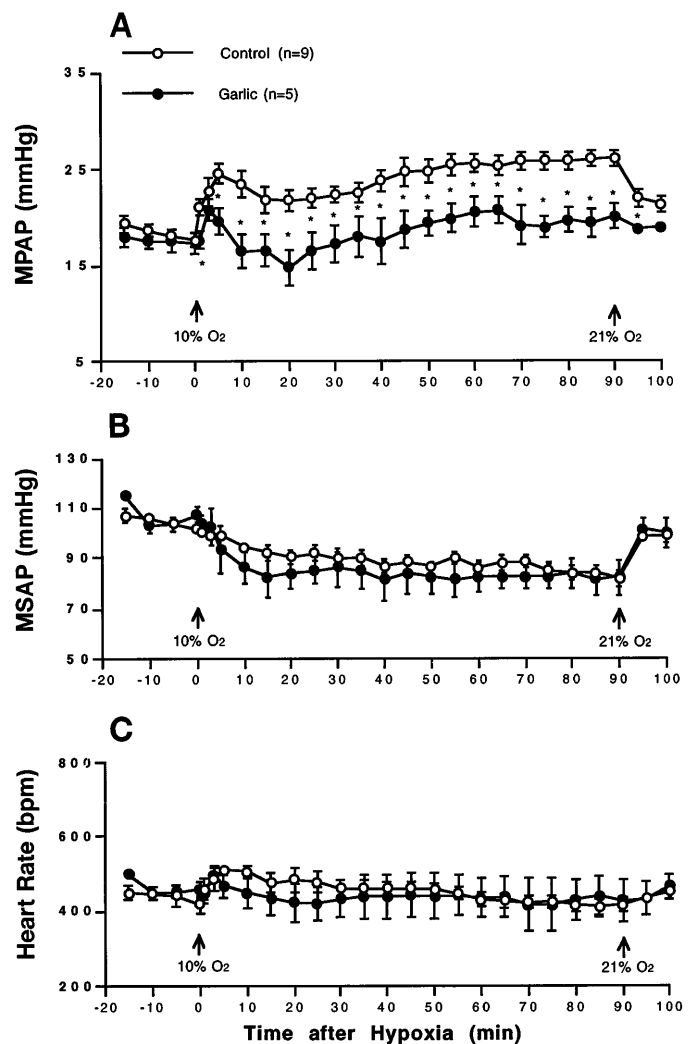


Fig. 1. Effects of garlic (100 mg · kg⁻¹ · day⁻¹, 1 gavage/day for 5 days) on mean pulmonary arterial pressure (MPAP; A), mean systemic arterial pressure (MSAP; B) and heart rate (C) in rats during short-term exposure to hypoxic conditions (10% O₂ for 90 min; left arrows). Right arrows, return to 21% O₂. bpm, Beats/min. Values are means ± SE; n, no. of rats. * *P* < 0.05.

creased transiently in the control animals after hypoxic exposure, but no significant differences in HR were observed between the control and garlic-treated animals (Fig. 1C). These findings demonstrate a protective effect of garlic gavage against the development of pulmonary hypertension in response to acute normobaric hypoxia.

Effects of garlic exposure on isolated pulmonary arterial segments. To determine whether garlic exerts a direct effect on the pulmonary vasculature, intralobar pulmonary arterial segments were isolated from untreated animals, and their responses to the garlic solution were assessed *in vitro*. Figure 2 depicts typical tracings of endothelium-intact pulmonary arterial ring responses to both acetylcholine and garlic solution. Figure 2A shows the rapid dose-dependent relaxation response to acetylcholine. Figure 2B shows the relaxant response to garlic solution (1–300 $\mu\text{g/ml}$). Exposure to garlic resulted in a significant dose-dependent vasorelaxation similar to acetylcholine. However, in contrast to acetylcholine, relaxation induced by garlic developed more slowly, with the average time for the maximum garlic-induced relaxant response being ~ 2 –3 min compared with 25–45 s for acetylcholine-induced relaxation. In addition, the effects of garlic were reversible with washout and reproducible with a second exposure to the garlic solution (data not shown), indicating a lack of direct toxicity on the vascular endothelium. These results document a direct effect of garlic administration on pulmonary arterial vascular tone.

Mechanism of garlic effect on isolated pulmonary arterial rings. To assess the endothelium dependence of the effect of garlic on the pulmonary arterial system, we repeated studies in isolated arterial segments after pretreatment with the specific nitric oxide synthase inhibitor L-NAME and after endothelium disruption. Pretreatment of endothelium-intact rat pulmonary arterial segments with L-NAME completely abolished the acetylcholine-induced relaxation as expected (Fig. 3),

and abolition of acetylcholine-induced relaxation was also used to ensure complete endothelial disruption in studies with mechanically denuded segments. L-NAME pretreatment resulted in an 80% inhibition of garlic-induced relaxation (Fig. 4). The pulmonary arterial relaxation response to 100 $\mu\text{g/ml}$ of garlic was reduced from -35 ± 2 ($n = 10$ rings) to $-7 \pm 1\%$ ($n = 12$ rings) in the presence of L-NAME. A similar reduction in garlic-induced relaxation was observed in endothelium-disrupted rat pulmonary arterial segments ($-16 \pm 2\%$; $n = 10$ rings). However, in both conditions, a degree of garlic-induced relaxation remained (20–30%) despite the inhibition of nitric oxide production or mechanical removal of the endothelium. Together, these data demonstrate that the effect of garlic on pulmonary arterial segments is predominately but not completely endothelium dependent and involves the production of nitric oxide.

DISCUSSION

In this study, we have demonstrated that garlic powder administered enterally prevents the acute hypoxic increase in pulmonary pressure in an animal model of pulmonary hypertension without causing significant effects on systemic blood pressure or heart rate. In addition, we show that garlic causes a significant dose-dependent relaxation of isolated rat intralobar pulmonary arteries predominantly through endothelium-dependent effects on pulmonary vascular smooth muscle. These studies suggest that garlic may prevent hypoxic pulmonary vasoconstriction through effects on the pulmonary arterial system.

This is the first *in vivo* animal study that has investigated the effects of garlic on the pulmonary vasculature in the setting of alveolar hypoxia. We utilized garlic powder manufactured from the dehydration of fresh garlic bulbs. In our animals with a metabolic rate two to three times that of humans, the

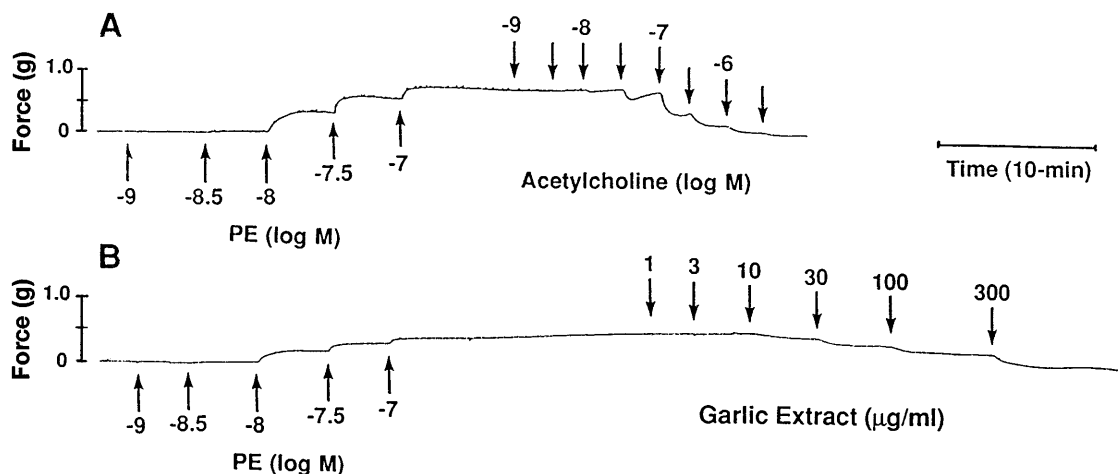


Fig. 2. Typical tracings of acetylcholine (A)- and garlic extract (B)-induced relaxation on isolated rat intralobar pulmonary arteries. Rat intralobar pulmonary arteries were isolated and pretreated with 5 μM indomethacin as described in METHODS. Initial contractile tone was induced with phenylephrine (PE) before addition of cumulative doses of either acetylcholine or garlic extract (arrows). Similar experiments were performed in at least 8–12 other different pulmonary arterial ring preparations.

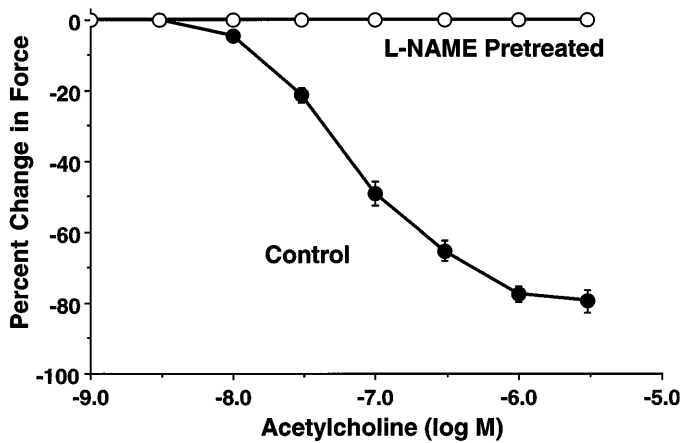


Fig. 3. Effects of N^G -nitro-L-arginine methyl ester (L-NAME) pretreatment on acetylcholine-induced endothelium-dependent relaxation in isolated rat intralobar pulmonary arteries. Pulmonary arteries were isolated and pretreated with $5 \mu\text{M}$ indomethacin as described in METHODS. For inhibition of endothelial nitric oxide synthesis, 0.3 mM L-NAME was added to incubation 20 min before preparations were tested with acetylcholine. Data are means \pm SE of 8–10 different pulmonary ring preparations obtained from 3–4 rats. Control rings relax in a normal fashion to acetylcholine, demonstrating presence of intact functional endothelium. This response is completely inhibited by L-NAME pretreatment. Abolition of acetylcholine response was also used to document complete endothelial removal in endothelium-disrupted ring studies (data not shown).

daily dose of garlic administered was approximately equivalent to a human consuming 2 g of garlic powder or 4 large garlic cloves. We chose to administer garlic by gavage for 5 days before performing hypoxic challenges to assess the effects in a relative “steady state” based on previous animal and human studies (9, 14, 17) that have demonstrated effects on vascular tone over 5–24 h after oral administration of garlic. The administration

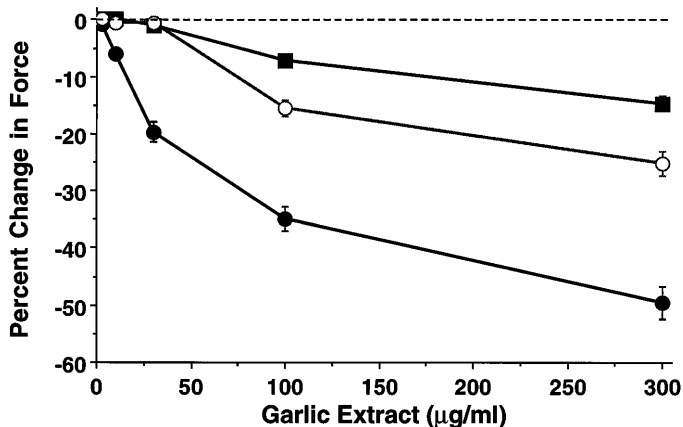


Fig. 4. Effects of L-NAME pretreatment and endothelium disruption on garlic extract-induced relaxation in isolated rat intralobar pulmonary arteries. Pulmonary arteries were isolated and pretreated with $5 \mu\text{M}$ indomethacin as described in METHODS. For inhibition of endothelial nitric oxide synthesis, 0.3 mM L-NAME was added to incubation 20 min before preparations were tested with garlic extract. For endothelium-disrupted group, intimal endothelium was denuded with a wooden applicator before being tested. ●, Vessels with intact endothelium; ○, vessels with disrupted endothelium; ■, vessels with intact endothelium pretreated with L-NAME. Data are means \pm SE of 8–12 different pulmonary ring preparations obtained from 4–5 different rats.

of our aqueous extract of garlic powder by gastric gavage to rats for 5 days before hypoxic exposure significantly inhibited hypoxic pulmonary vasoconstriction in all animals and thereby preserved normal mean pulmonary pressures. In contrast, all control animals had significant elevations in pulmonary pressures in response to low alveolar oxygen tension. The observation that MSAP and HR in the garlic-fed animals did not differ from MSAP and HR in the control animals is most consistent with a preferential effect on the pulmonary vasculature. Other studies have demonstrated a modest decrease in systemic blood pressure in animals (2, 4) and humans (1, 7, 10, 19) receiving garlic, although this effect has not been uniformly observed (16). Marked differences in dosage and route of garlic administration, differences in methods of preparation (water, oil, or ethanol extracts vs. rehydrated garlic powder or fresh garlic) and differences in the timing of the assessment of effects on systemic pressure in these studies may have influenced the components of garlic which were evaluated and the magnitude of effects observed.

Our aqueous extract of garlic powder causes a significant dose-dependent relaxation in intralobar pulmonary arterial rings both in the presence and absence of nitric oxide synthase blockers and intact endothelium. This observation indicates that at least two mechanisms contribute to the vasodilatory effect. First, L-NAME pretreatment of endothelium-intact intralobar pulmonary arteries resulted in an 80% inhibition of garlic-mediated vasodilatation, establishing that a major component of the garlic effect on smooth muscle is mediated through the production of nitric oxide in endothelial cells. This finding is confirmed by a corresponding decrease in the effect of garlic in endothelium-disrupted pulmonary arterial segments and is consistent with recent work (6) demonstrating that garlic administration may result in activation of calcium-dependent endothelial nitric oxide synthase in certain tissues. Second, a degree of garlic-induced vasorelaxation was maintained in both L-NAME-treated intact pulmonary arterial segments and endothelium-disrupted pulmonary arterial segments, indicating an additional direct effect on vascular smooth muscle cells. This finding is compatible with studies in isolated vascular strips that demonstrate that garlic can induce vasodilatation via smooth muscle cell membrane hyperpolarization and/or inhibition of the opening of calcium channels (18).

The components in garlic that influence vascular tone are not well characterized. Much interest has focused on the numerous sulfur-containing compounds that appear to be important for a number of the medicinal actions of garlic (3). Cysteine sulfoxides such as alliin, which are transformed into thiosulfinates, comprise a significant portion of these sulfur compounds (16). Intra-arterial administration of synthesized alliin (2-propenethiosulfinate), the predominant thiosulfinate in garlic, resulted in mesenteric arterial vasodilatation in the cat (13). Ku et al. (12) initiated and reported on preliminary studies focused on deter-

mining the role of allicin in altering pulmonary vascular tone by assessing the vasoactive efficacy of a number of garlic preparations with varying allicin yields. The degree of pulmonary vasorelaxation correlated directly with the allicin yield in each preparation. In addition, we have recently investigated a specially prepared garlic powder in which alliin (kindly provided by Dr. Larry Lawson, Murdock, Madaus, Schwabe Group; Springville, UT), the parent compound of allicin, was removed. This specific preparation of garlic had no effect on pulmonary vascular tone (Ku, unpublished data). Taken together, these studies suggest that allicin and/or other thiosulfinates derived from alliin are important mediators of the vasorelaxation induced by garlic in isolated pulmonary arterial segments. Future studies are needed to determine the molecular mechanisms by which thiosulfinates cause vasorelaxation in pulmonary arterial segments and to define whether the effects observed in isolated pulmonary arterial segments in vitro account for the alterations observed in the entire pulmonary vasculature in vivo.

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