ELSEVIER

European Journal of Pharmacology 276 (1995) 21–26

ejp

Analysis of responses of allicin, a compound from garlic, in the pulmonary vascular bed of the cat and in the rat

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Received 25 October 1994; revised 14 December 1994; accepted 29 December 1994

Abstract

Allicin, diallyl disulfide-oxide, an active ingredient released from garlic is a systemic vasodilator that acts by an unknown mechanism. In the present experiments, pulmonary vascular responses to allicin (0.1–1.0 mg) were studied in the intact-chest anesthetized cat and in the isolated lung of the rat under constant flow conditions. When baseline tone in the pulmonary vascular bed of the cat was raised with U46619 (11α,9α-epoxymethano-9α,11β-dideoxyprostaglandin $F_{2\alpha}$), intralobar injections of allicin produced dose-related decreases in pulmonary arterial pressure without changing left atrial pressure indicating that allicin had significant vasodilator activity in the pulmonary vascular bed when tone was increased experimentally. Allicin also decreased systemic arterial pressure in a dose-related manner. In terms of relative vasodilator activity in the cat, allicin was 100-fold less potent than sodium nitroprusside and many orders of magnitude less potent than isoproterenol. In the cat, vasodilator responses to allicin were unchanged by methylene blue or N^{ω}-nitro-L-arginine methyl ester. Allicin also significantly diminished the pulmonary pressor response to ventilatory hypoxia in the isolated perfused rat lung. These data show that allicin has significant vasodilator activity in the pulmonary vascular bed of the cat and the rat. The present data suggest that pulmonary vasodilator responses to allicin are independent of the synthesis of endothelial-derived relaxing factor or the activation of soluble guanylate cyclase.

Keywords: Allicin; Pulmonary vascular bed, rat; Pulmonary vascular bed, cat; Vasodilator response; N^{ω}-Nitro-L-arginine methyl ester; Methylene blue; Hypoxic pulmonary vasoconstriction; Guanylate cyclase

1. Introduction

While garlic extracts have been used in the treatment of a wide range of disorders in the past, the mechanism of action is unclear. Allicin is a compound released from garlic that is formed naturally through the action of the enzyme, alliinase, on the parent compound, alliin, when the tissue of the garlic bulb is disrupted (Stoll and Seebeck, 1948). Ether extracts of garlic (*Allium sativum*) and partially purified distilled extracts of garlic have been reported to inhibit human platelet aggregation in vitro (Apitz-Castro et al., 1983). The inhibition of human platelet aggregation does not involve an effect on cyclooxygenase or thromboxane synthase activity or on cyclic adenosine monophosphate (AMP) levels (Mayeux et al., 1988). Garlic extracts have also been reported to be antibacterial (Cavillito and Bailey, 1944), antifungal (Yoshida et al., 1987), antiviral (Weber et al., 1992), and antiprotozoal (Mirelman et al., 1987). Allicin has been shown to have antimutagenic activity in adenocarcinoma cell lines (Shalinsky et al., 1989) and to reduce mutagenesis in Salmonella tester strains (Knasmuller et al., 1989). Garlic extracts have inhibitory effects on cholesterol biosynthesis (Bordia et al., 1977). Previous studies in the feline mesenteric vascular bed have demonstrated that allicin induces vasodilation independent of β-adrenoceptor activation or by stimulating the formation of cyclooxygenase products (Mayeux et al., 1988). Recently, a garlic containing preparation containing

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SSDI 0014-2999(94)00784-5

1.3% alliin in a modest dose (14 mg of allicin) was shown to cause significant decreases in diastolic blood pressure in severely hypertensive patients (McMahon and Vargas, 1993). Although vasodilator responses to allicin have been described in the mesenteric vascular bed, little if anything, is known about responses to allicin in the pulmonary vascular bed. The present study was therefore undertaken to investigate responses to allicin in the pulmonary vascular bed of the intact-chest cat under constant flow conditions when tone was increased with the thromboxane A_2 mimic, U46619 (11α,9α-epoxymethano-9α,11β-dideoxyprostaglandin $F_{2\alpha}$) and in the rat when tone was increased by ventilatory hypoxia.

2. Materials and methods

Twenty-two mongrel cats of either sex weighing 3.0–4.6 kg were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and were anesthetized with pentobarbital sodium (30 mg/kg i.v.). The animals were restrained in the supine position on a fluoroscopic table and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 100% oxygen. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from a femoral artery, and i.v. injections were made into a catheter positioned in the inferior vena cava from a femoral vein.

For perfusion of the left lower lung lobe a specially designed 28-cm triple-lumen 6F balloon perfusion catheter (Arrow International, Reading, PA) was passed under fluoroscopic guidance from an external jugular vein into the artery to the left lower lung lobe. After the animal had been heparinized (1000 units/kg i.v.), the lobar artery was isolated by distension of the balloon cuff on the perfusion catheter, and the lobe perfused with a perfusion pump (Model 1210, Harvard Apparatus, South Natick, MA) by way of the catheter lumen beyond the balloon cuff with blood withdrawn from a femoral artery. The perfusion rate was adjusted so that lobar arterial perfusion approximated mean pressure in the main pulmonary artery and was not changed during an experiment. The perfusion rate ranged from 25–45 ml/min and lobar arterial pressure was measured from a catheter port 5 mm from the tip of the perfusion catheter. Left atrial pressure was measured with a radioopaque 6F single lumen or 6F double lumen catheter passed transseptally into the vein draining the left lower lobe. The catheter tip was positioned so that the left atrial pressure port on the distal lumen was 1–2 cm into the lobar vein and the second catheter port was near the venoatrial junction. When necessary, blood could be withdrawn or infused through this second catheter lumen to maintain left atrial pressure constant. All vascular pressures, measured with Spectramed DTX Plus transducers (Viggo-Spectromed, Oxnard, CA) zeroed at right atrial level. Mean vascular pressures obtained by electronic averaging were recorded (Model 7 recorder, Grass Instrument Co., Quincy, MA).

Methylene blue (American Regents, Co., Shirley, NY and Sigma Chemical Co., St. Louis, MO) and N^{ω}-nitro-L-arginine methyl ester hydrochloride (Sigma) were dissolved in normal saline immediately before use. Acetylcholine chloride, sodium nitroprusside, and isoproterenol hydrochloride (Sigma) were dissolved in normal saline. Allicin was chemically synthesized within our lab. Allicin was prepared by hydrogen peroxide oxidation of fractionally distilled diallyl disulfide. It was dissolved in water and kept frozen (Mayeux et al., 1988). The thromboxane receptor agonist, U46619, (11α,9α-epoxymethano-9α,11β-dideoxyprostaglandin $F_{2\alpha}$; Upjohn Co., Kalamazoo, MI) was dissolved in 100% ethanol at a concentration of 10 mg/ml, and further dilutions were made in normal saline. Working solutions were prepared on a frequent basis (every 2 or 3 days) by diluting the stock solution in 0.9% NaCl solution, were stored in brown, stoppered bottles, and were kept on crushed ice during experiments.

Because the pulmonary vascular bed of the intact-chest cat has little, if any, vasoconstrictor response under when the F_IO_2 is greater than 0.21, pulmonary arterial pressure in the bed must be actively increased so vasodilator responses can be expressed. In all experiments, tone was raised in the control period to an average value of 35 ± 3 (32–40 mm Hg) with an intralobar infusion of U46619. Pulmonary vascular responses of allicin, sodium nitroprusside and isoproterenol were compared when tone (lobar arterial pressure) was increased to 32–40 mm Hg with the thromboxane mimic U46619 infused at rates of 40–340 ng/min. The agonists were injected in small volumes directly into the perfusion circuit distal to the pump in a random sequence during the control period when tone was increased with U46619. Afterwards, the U46619 infusion was terminated, and lobar arterial pressure was permitted to return to near control values. After the peak increase in lobar arterial pressure in response to N^{ω}-nitro-L-arginine methyl ester was attained, the U46619 infusion was resumed if necessary to raise pulmonary vascular tone to a level similar to that attained during the control period (32–40 mm Hg). In some experiments, however, N^{ω}-nitro-L-arginine methyl ester administration alone was sufficient to raise lobar vascular tone to a level equal to the control level, and in these experiments, U46619 infusion was resumed only later, when lobar arterial pressure had fallen below 32 mm Hg.

In separate experiments with methylene blue, responses to allicin were again studied during the control period when tone was increased with U46619. The U46619 infusion was terminated and lobar arterial pressure was allowed to return to near control values. An intralobar infusion of methylene blue (0.4–1.2 mg/min i.a.) was begun and the infusion rate was gradually increased to a final rate of 2.8 ± 0.6 mg/min at which time lobar arterial pressure had attained an average value of 35 ± 3. After the peak increase in lobar arterial pressure in response to methylene blue, the U46619 infusion was resumed if necessary to raise pulmonary vascular tone to a level similar to that attained during the control period. In some experiments, methylene blue administration alone was sufficient to raise lobar vascular tone to a level equal to the control level, and in these experiments, U46619 infusion was resumed only later, when lobar arterial pressure had fallen below 32 mm Hg.

In experiments in which the effects of allicin were investigated in the isolated lung of the rat, six male Sprague-Dawley rats weighing 300–350 g (Hill Top Laboratories, Scottdale, PA) used in these experiments were intraperitoneally anesthetized with pentobarbital sodium (50 mg/kg). After stable anesthesia was obtained, the trachea was surgically approached, cannulated with a short section of polyethylene tubing, connected to a rodent ventilator (Harvard Apparatus, South Natick, MA) and ventilated with room air enriched with 95% O_2-5% CO_2, with a tidal volume of 5–7 ml/kg and 2 cm H_2O positive end-expiratory pressure. The rats were heparinized with 1000 units of heparin i.v. (Sigma Chemical Co., St. Louis, MO) and were rapidly exsanguinated by withdrawing blood from the carotid artery.

The lungs were exposed by median sternotomy, and a ligature was placed around the aorta to prevent systemic loss of blood. The main pulmonary artery was catheterized, and the lungs were removed en-block and suspended in a warmed (38°C), humidified (100%) water-jacketed chamber. An external heat exchanger (Haake D1 Heat Exchanger, Baxter Instrument Co., Harahan, LA) maintained the temperature of the perfusate and the isolated lung chamber constant throughout the experiment. The perfusate solution (15 ml of heparinized blood and 5 ml modified Krebs-Heinseleit solution) was placed in a reservoir and constantly mixed by a magnetic stirrer (Thermolyne, Cimarec II, Dubuque, IA). The lungs were perfused with a peristaltic roller pump (Cole-Parmer Instrument Co., Berrington, IL). Once the isolated lung perfusion circuit was established, the flow rate was set at 8–14 ml/min to maintain physiologic baseline pulmonary arterial perfusion pressure of 15 ± 0.5 mm Hg. The flow rate was confirmed in some experiments by timed collection of blood using a stop watch and graduated cylinder at the end of the experiment. All vascular pressures were measured with Viggo-Spectramed transducers (Viggo-Spectramed, Oxnard, CA) zeroed at the level of the pulmonary arterial cannula. Pulmonary arterial perfusion pressure, airway pressure, and reservoir blood level were continuously monitored, electronically averaged, and recorded with a Grass polygraph, Model 7 (Grass Instrument Co., Quincy, MA). The modified Krebs-Heinseleit solution had the following composition (g/l): NaCl, 66.37; KCl, 3.58; $CaCl_2 \cdot 2H_2O$, 3.68; KH_2PO_4, 1.63; $MgSO_4 \cdot 7H_2O$, 1.45; $NaHCO_3$, 2.0; Ficoll (type 70, Sigma Chemical Co., St. Louis, MO), 2.0; (pH 7.35–7.45). The solution was made fresh daily in double distilled water.

Blood gases and pH were measured with an Instrumentation Laboratory Model Micro 13 analyzer. All hemodynamic data are expressed in absolute units and are presented as means $\pm$ S.E.M. Responses represent peak changes, unless otherwise noted. The data were analyzed using a paired t-test (StatView, Abacus Concepts, Berkley, CA) completed on a MacSE/30. A value of $P < 0.05$ was used as the criterion for statistical significance.

3. Results

The effects of allicin on the pulmonary vascular bed of the intact-chest cat are illustrated in Fig. 1. When baseline tone (lobar arterial pressure) in the pulmonary vascular bed was raised to a high steady value (32–40 mm Hg) with an infusion of U46619, injections of allicin into the perfused lobar artery in doses of 0.1–1.0 mg, caused significant dose-related decreases in lobar arterial pressure without changing left atrial pressure (Fig. 1). Systemic arterial pressure was decreased in a dose-related manner (Fig. 1).

The relative vasodilator actions of allicin, sodium nitroprusside and isoproterenol in the pulmonary vascular bed are compared in Fig. 2. Under elevated tone conditions when lobar arterial pressure was increased with U46619, intralobar injections of the three vasodilator agents caused significant dose-related decreases in lobar arterial pressure (Fig. 2). When comparisons were made on a nmol basis, sodium nitroprusside was approximately 100-fold more potent than allicin, and isoproterenol was approximately four orders of magnitude more potent than allicin in dilating the lobar vascular bed of the intact-chest cat (Fig. 2).

The effects of the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (100 mg/kg i.v.) on pulmonary vasodilator responses to allicin are summarized in Fig. 3. Decreases in lobar arterial pressure in response to intralobar injections of allicin were not significantly changed after administration of N^{ω}-nitro-L-arginine methyl ester (Fig. 3, upper left panel). De-

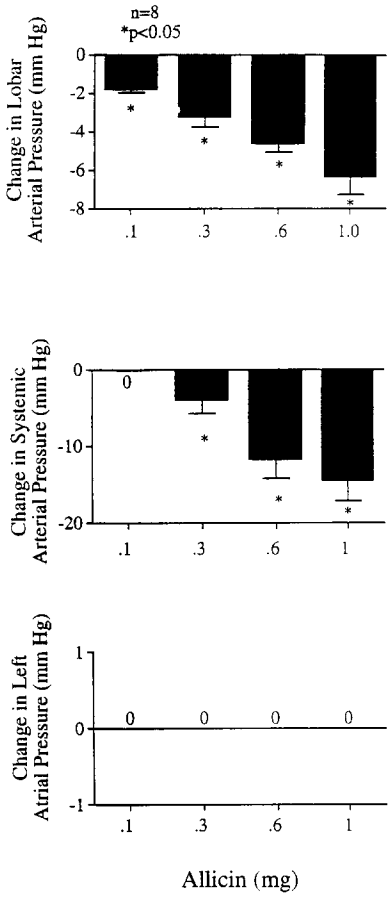

Fig. 1. Changes in lobar arterial pressure, systemic arterial pressure, and in left atrial pressure in response to intralobar injections of allicin in doses of 0.1–1.0 mg into the perfused lobar artery in the cat. Tone in the pulmonary vascular bed was raised with an infusion of U46619 into the perfused lobar artery.

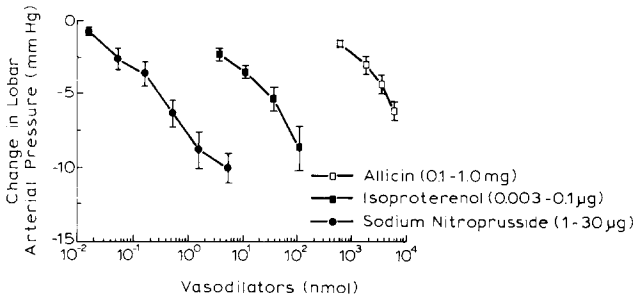

Fig. 2. Comparison of the decreases in lobar arterial pressure in response to intralobar injections of allicin, isoproterenol, and sodium nitroprusside in the cat. The doses of the vasodilator agents were compared on a nmol basis. *n* indicates number of animals. The asterisk indicates that responses are significantly different from control. Tone in the pulmonary vascular bed was raised with an infusion of U46619 into the perfused lobar artery and was similar in experiments in which responses to allicin, isoproterenol and sodium nitroprusside were compared.

creases in lobar arterial pressure in response to intralobar injections of acetylcholine were significantly reduced after the nitric oxide synthase inhibitor was administered (Fig. 3). Decreases in lobar arterial pressure in response to nitric oxide and sodium nitroprusside were enhanced significantly following treatment with the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (Fig. 3, upper right panel).

The effects of methylene blue on pulmonary vasodilator responses to allicin are also summarized in Fig. 3. When lobar arterial pressure was increased by infusion of methylene blue, decreases in lobar arterial pressure in response to intralobar injections of allicin were not significantly different from the values ob-

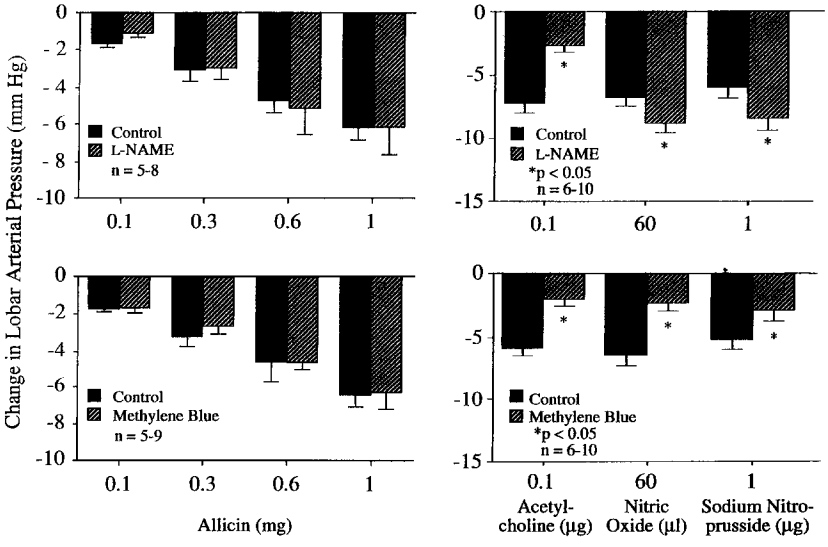

Fig. 3. Influence of N^{ω}-nitro-L-arginine methyl ester and methylene blue on decreases in lobar arterial pressure in response to allicin (left panels), and acetylcholine, nitric oxide and sodium nitroprusside (right panels) under elevated tone conditions. *n* indicates number of animals. The asterisk indicates that responses are significantly different from control.

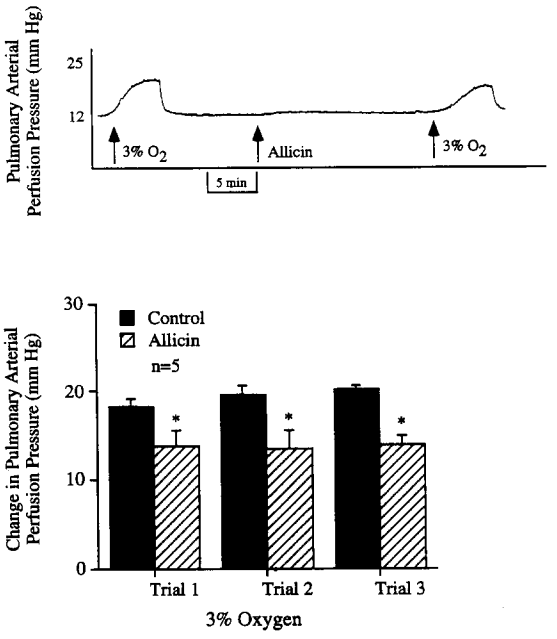

Fig. 4. Upper panel. Tracing from an experiment demonstrating the pulmonary arterial pressor response when the lung was challenged with 3% 0_2-5%CO_2 before and after injection of allicin into the perfusion reservoir. Lower panel. Influence of allicin on the pulmonary arterial pressor response to ventilatory hypoxia. *n* indicates number of animals. The asterisk indicates that responses are significantly different from control.

tained when allicin was injected during the control U46619 infusion (Fig. 3, lower left panel). During infusion of methylene blue, decreases in lobar arterial pressure in response to intralobar injections of acetylcholine, nitric oxide and sodium nitroprusside were significantly decreased when compared with responses obtained when these agents were injected during the control U46619 infusion (Fig. 3).

The effects of allicin on the pressor response to ventilatory hypoxia were investigated in the isolated perfused rat lung and these data are summarized in Fig. 4. During the control period ventilation with the hypoxic gas mixture (3% 0_2-5% $C0_2$-92% N_2) for 5–8 min, increased pulmonary arterial perfusion pressure from 15 ± 0.5 to 33 ± 1 mm Hg (Δ 18 ± 0.4 mm Hg) and the response was reproducible when the lung was challenged at intervals of 10–15 min (Fig. 4, lower panel). After hypoxic ventilation was terminated, the lung ventilated with 95% O_2-5% CO_2, and tone was allowed to return to baseline value. At this time, allicin was then added into the perfusion reservoir and allowed to circulate for 15 min. When pulmonary arterial perfusion pressure had attained a steady level after administration of allicin, the lung was rechallenged with the hypoxic gas mixture for 5–8 min. Allicin significantly diminished the pulmonary pressor response to ventilatory hypoxia (Fig. 4, lower panel).

4. Discussion

Results of the present investigation show that allicin decreases lobar arterial pressure when tone in the pulmonary vascular bed is increased to a high steady level with U46619 in the cat. In as much as pulmonary blood flow was maintained constant and left atrial pressure was unchanged, the decreases in pulmonary lobar arterial pressure reflect decreases in pulmonary lobar vascular resistance. The decreases in lobar arterial pressure were dose-dependent and were not altered by N^{ω}-nitro-L-arginine methyl ester or methylene blue. These results suggest that decreases in pulmonary vascular resistance in response to allicin appear to be independent of the release of endothelium-derived NO or of activation of soluble guanylate cyclase.

The observation that pulmonary vasodilator responses to acetylcholine were decreased indicates that nitric oxide release was inhibited by the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester treatment. Inhibition of nitric oxide synthase has been shown to enhance the sensitivity of soluble guanylate cyclase to nitric oxide and this may account for the observed increases in response to sodium nitroprusside and to nitric oxide following treatment with N^{ω}-nitro-L-arginine methyl ester in our studies (Moncada et al., 1991a). Treatment with methylene blue reduced vasodilator responses to acetylcholine, nitric oxide and sodium nitroprusside. The observed effects of methylene blue on responses to acetylcholine which releases nitric oxide from the endothelium and sodium nitroprusside which releases nitric oxide are consistent with previous observations with methylene blue and the proposed actions of methylene blue on soluble guanylate cyclase (McMahon and Kadowitz, 1992; Moncada et al., 1991b).

Although the mechanism by which allicin dilates the pulmonary vascular bed is uncertain, this substance has significant pulmonary vasodilator activity. In terms of relative vasodilator activity, allicin is approximately 100-fold less potent that sodium nitroprusside and many orders of magnitude less potent than isoproterenol in the pulmonary vascular bed. Allicin produced dose-related decreases in vascular resistance in the mesenteric vascular bed of the cat and these responses were not significantly changed in the presence of meclofenamate or propranolol, suggesting that allicin dilates the mesenteric vascular bed by a mechanism independent of activation of β-adrenoceptors or formation of cyclooxygenase products (Mayeux et al., 1988). Moreover, allicin produced a concentration-dependent inhibition of human platelet aggregation in vitro without affecting cyclooxygenase or thromboxane synthase activity or cyclic adenosine monophosphate (AMP) levels (Mayeux et al., 1988). There are no known antagonists which inhibit the pulmonary or systemic vasodilator effects of

this constituent of garlic. The nature or type of allicin receptors in resistance vessel elements in the pulmonary vascular bed are unknown at the present time and require future studies.

The present data also show that when pulmonary vascular tone is increased with hypoxia, allicin reduces the pressor response to hypoxia in the isolated rat lung. These data suggest that allicin may be useful in the treatment of pulmonary hypertensive disorders in which hypoxia is a prominent component such as in chronic obstructive pulmonary disease. The present data suggest other allicin analogues should be developed for clinical use since allicin has vasodilator efficacy and appears to lack toxicity. Allicin has been found to be immediately converted to allyl mercaptan in whole blood (Lawson and Wang, 1993) as well as to react rapidly with blood proteins (Lawson et al., 1992) and pilot data in our laboratory indicate that these purified analogues possess significant pulmonary vasodilator activities in the rat and the cat.

In conclusion, results of the present study show that allicin has significant vasodilator activity in the pulmonary vascular bed of the cat and the rat when tone is increased experimentally. Although the mechanism by which allicin induces vasodilatation or diminishes hypoxic pulmonary vasoconstriction is uncertain, the results of the present investigation suggest that this novel constituent from garlic may be useful in the treatment of pulmonary hypertensive disorders including primary pulmonary hypertension and chronic obstructive pulmonary disease in which hypoxia is a prominent feature.

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