Analysis of responses of garlic derivatives in the pulmonary vascular bed of the rat

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1Departments of Anesthesiology and Pharmacology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430; and 2Departments of Anesthesiology and Pharmacology, Tulane University Medical Center, New Orleans, Louisiana 70112

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Kaye, Alan D., Bracken J. De Witt, Muhammad Anwar, Donald E. Smith, Chang J. Feng, Philip J. Kadowitz, and Bobby D. Nossaman. Analysis of responses of garlic derivatives in the pulmonary vascular bed of the rat. J Appl Physiol 89: 353–358, 2000.—Allicin, an extract from garlic, has been shown to be a systemic and pulmonary arterial vasodilator that acts by an unknown mechanism. In the present experiments, pulmonary vascular responses to alliin (10–100 μg), allyl mercaptan (0.3–1 mg), and diallyl disulfide (0.3–1 mg) were studied in the isolated lung of the rat under constant-flow conditions. When baseline tone in the pulmonary vascular bed of the rat was raised to a high-steady level with the thromboxane A2 mimic U-46619, dose-related decreases in pulmonary arterial pressure were observed. In terms of the mechanism of action of allicin vasodilator activity in the rat, responses to alliin were not significantly different after administration of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester, the KATP channel antagonist U-37883A, or the cyclooxygenase inhibitor sodium mcelofenamate, or when lung ventilation was interrupted. These data show that alliin has significant vasodilator activity in the pulmonary vascular bed of the rat, whereas allyl mercaptan and diallyl disulfide produced no significant changes in pulmonary arterial perfusion pressure. The present data suggest that pulmonary vasodilator responses to alliin are independent of the synthesis of nitric oxide, ATP-sensitive K+ channels, activation of cyclooxygenase enzyme, or changes in bronchomotor tone in the pulmonary vascular bed of the rat.

alliin; allyl mercaptan; diallyl disulfide; lung circulation

THE FIRST DETAILED DESCRIPTION of garlic (Allium sativum) as an herbal medicinal plant was written by Pedanius Dioscorides in the first century (19). Since that time, garlic and its extracts have been used in the treatment of a wide range of disorders. However, the mechanism behind the action of garlic remains unclear. Garlic bulb cells contain the cysteine sulfoxide alliin, which is effective protection against microorganisms underground (19). When garlic is crushed, cut, chewed, or attacked by a microbe, alliin is exposed to the vacuolar enzyme alliinase, which forms the thiosulfinate alliin. Allicin is an odorless, reactive intermeiate species that can be transformed into a variety of compounds depending on the environmental conditions.

Allicin, when exposed in heated aqueous solution, forms a lipid-soluble oligosulfide known as diallyl disulfide. It has been suggested that garlic intake can modify the risk of colon cancer to women, because diallyl disulfide is an effective inhibitor of the growth of neoplastic CMT-13 cells and of N-acetyltansferase activity in the human colon adenocarcinoma cell line (6, 20). Furthermore, diallyl disulfide has been shown to be an effective inhibitor for the promotion phase of 9,10-dimethyl-1,2-benzanthracene-induced skin tumors in the mouse (2).

Allyl mercaptan is an odorant compound that is the main component of “garlic breath” after eating garlic cloves. Allyl mercaptan is quantitatively formed from alliin or diallyl disulfide with cysteine via the intermediate compound S-allylmercaptocysteine when it comes in contact with whole blood (17). Allyl mercaptan, or a further metabolite of it, may be the major means of the pharmacological (nontopical, nonenteral) action of alliin or diallyl disulfide (17).

Extracts of garlic have been reported to inhibit human platelet aggregation in vitro (1). The inhibition of human platelet aggregation does not involve an effect on cyclooxygenase, thromboxane synthase activity, or on adenosine 3',5'-cyclic monophosphate levels (14). Garlic extracts have also been reported to be antibacterial (5), antifungal (22), antiviral (21), and antiprotozoal (16) and to have inhibitory effects on cholesterol biosynthesis (3). Allicin has been shown to have antimutagenic activity in adenocarcinoma cell lines (18) and to reduce mutagenesis in Salmonella tester strains (13). 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 (14). Recently, an alliin-containing preparation was shown to cause significant decreases in diastolic blood pressure in severely hypertensive patients (15). Allicin...
also has been shown to prevent hypoxic pulmonary hypertension in rats; however, the mechanism by which allicin produces this effect is unknown (10, 12). Therefore, the purpose of the present study was to investigate responses to allicin, diallyl disulfide, and allyl mercaptan in the pulmonary circulation of the rat under constant-flow conditions.

MATERIALS AND METHODS

Thirty-five male Sprague-Dawley rats weighing 300–350 gm (Hill Top Laboratories, Scottdale, PA) were anesthetized with pentobarbital sodium (50 mg/kg body wt, ip). 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% O₂-5% CO₂ for all experimental periods, with a tidal volume of 5–7 ml/kg and 2 cmH₂O positive end-expiratory pressure. The rats were heparinized with 1,000 U iv of heparin (Sigma Chemical, St. Louis, MO) and 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 bloc and suspended in a warmed (38°C), humidified (100%) water-jacketed chamber. An external heat exchanger (Haake D1 Heat Exchanger, Baxter Instrument, Harahan, LA) maintained constant temperature of the perfusate and the isolated lung chamber throughout the experiment. The perfusate solution (15 ml of heparinized blood and 5 ml modified Krebs-Henseleit 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, Berrington, IL). Once the isolated lung perfusion circuit was established, the flow rate was set at 8–14 ml/min to maintain a physiologic baseline pulmonary arterial perfusion pressure of 12 ± 1 mmHg. The flow rate was confirmed in some experiments by performing timed blood collection of blood with a stopwatch and a graduated cylinder at the end of the experiment. 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 model 7 polygraph (Grass Instrument, Quincy, MA). The modified Krebs-Henseleit solution had the following composition (g/l): 66.37 NaCl, 3.58 KCl, 3.68 CaCl₂ · 2H₂O, 1.63 KH₂PO₄, 1.45 MgSO₄ · 7H₂O, 2.0 NaHCO₃, 2.0 Ficoll (type 70, Sigma Chemical, St. Louis, MO) (pH = 7.35–7.45). The solution was made fresh daily in double distilled water.

N⁷-nitro-l-arginine methyl ester hydrochloride (Sigma, St. Louis, MO) was dissolved in normal saline immediately before use. Acetylcholine chloride, sodium arachidonate (Sigma, St. Louis, MO), sodium meclofenamate (Warner Lambert-Parke Davis, Ann Arbor, MI), adrenomedullin (Pep-tides International, Belmont, CA), calcitonin-gene related peptide (Bachem Bioscience, Philadelphia, PA), and U-37883A (Upjohn, Kalamazoo, MI) were dissolved in normal saline. Levcromakalim (SmithKline Beecham, Sussex, UK) was dissolved in 20% ethanol-saline solution at a concentration of 1 mg/ml and diluted 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 (14). Diallyl disulfide was dissolved in a solution of normal saline and 10% Tween 80. Allyl mercaptan was dissolved in normal saline. The thromboxane receptor agonist U-46619 (9,11-dideoxy-9α,11α-epoxymethanoprostaglandin F₂α) (Upjohn, 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 by diluting the stock solution in 0.9% NaCl solution, stored in brown, stoppered bottles, and kept on crushed ice during the studies.

Blood gases and pH were measured with an Instrumentation Laboratory model Micro 13 analyzer. To serve as a control before agonist injections, equivalent volumes of saline or distilled water were injected directly into the perfusion circuit. All injections were made in small volumes, in a random sequence, and sufficient time was permitted between agonist injections for pressures to return to baseline values. Because pulmonary blood flow and outflow pressure were maintained constant, changes in perfusion pressure in this preparation reflect changes in pulmonary vascular resistance. All vascular pressures are expressed in absolute units (mmHg) as means ± SE. The data were analyzed using a paired t-test (StatView, Abacus Concepts, Berkeley, CA). A value of P < 0.05 was used as the criterion for statistical significance.

U-46619 was infused to raise the pulmonary arterial pressure from a mean of 12 ± 1 to 35 ± 3 mmHg. Once a stable baseline was achieved, the effects of increasing doses of 1) allicin, 2) diallyl disulfide, and 3) allyl mercaptan were measured.

The pulmonary vascular bed of the rat is at a low resting tone when the inspired O₂ fraction is >0.21, thus pulmonary arterial pressure must be actively increased so that vasodilator responses can be expressed. In the present studies, tone was raised in the control period with 300–600 ng of U-46619. Under conditions of elevated tone in the control period, the pulmonary vascular responses of each agonist were obtained. Lungs were randomly assigned to one of four agonist groups: group A, N⁷-nitro-l-arginine methyl ester; group B, sodium meclofenamate; group C, U-37883A; and group D, main bronchus occlusion. The agonists were injected in small volumes directly into the perfusion circuit, distal to the pump, during the control period. Afterward, the antagonists were administered, and the agonists were again injected in a random sequence. Because N⁷-nitro-l-arginine methyl ester increases tone, U-46619 infusion was initially terminated when the nitric oxide synthase inhibitor was administered. After the peak increase in pulmonary arterial pressure in response to N⁷-nitro-l-arginine methyl ester (100 mg/kg ia) was attained, the U-46619 infusion was resumed as necessary to raise pulmonary vascular tone to a level similar to that attained during the control period. In some experiments, however, N⁷-nitro-l-arginine methyl ester administration alone was sufficient to raise pulmonary vascular tone to a level equal to that of the control, and, in these experiments, U-46619 infusion was only resumed when lobar arterial pressure became <30 mmHg.

In separate experiments with sodium meclofenamate and U-37883A, responses to the agonists were again studied during the control period with U-46619. Before infusion of sodium meclofenamate or U-37883A, U-46619 infusion was terminated, and pulmonary arterial pressure was permitted to return to near-control values. After the peak increase in pulmonary arterial pressure in response to sodium meclofenamate (2.5 mg/kg ia) or U-37883A (2 mg/kg ia) was achieved, U-46619 infusion was resumed, if necessary, to raise pulmonary vascular tone to a level similar to that
attained during the control period. In some experiments, sodium meclofenamate administration alone was sufficient to raise pulmonary vascular tone to a level equal to the control level, and, in these experiments, U-46619 infusion was resumed only if pulmonary arterial pressure became <30 mmHg.

In experiments in which the effects of lung ventilation on responses to allicin were investigated, responses were compared when the lung was ventilated and when ventilation was interrupted. In these experiments, airflow to the lung was interrupted at end-expiration by turning off the ventilator and clamping the tubing to the main bronchus. Pulmonary vascular responses were investigated under high-tone conditions beginning ~5 min after main bronchus occlusion.

**RESULTS**

**Responses to garlic compounds.** Responses to allicin, diallyl disulfide, and allyl mercaptan were compared in the isolated pulmonary vascular bed of the rat, and these data are summarized in Figs. 1 and 2. When baseline tone in the pulmonary vascular bed was raised to a high-steady value (35 ± 3 mmHg) with an infusion of U-46619, doses of 10–100 μg administration of allicin caused significant dose-related decreases in pulmonary arterial perfusion pressure (Fig. 1A). To determine relative vasodilator activity in the pulmonary vascular bed, doses of the compounds required to decrease pulmonary arterial perfusion pressure by 3 mmHg were compared. Calcitonin gene-related peptide was found to be significantly more potent than adrenomedullin and allicin, whereas allicin was not different in potency than adrenomedullin (Fig. 1B). In contrast, 0.3 and 1 mg injections of both diallyl disulfide and allyl mercaptan produced no significant changes in pulmonary arterial perfusion pressure. These data are summarized in Fig. 2.

**Influence of N\textsubscript{\textsuperscript{ω}}-nitro-L-arginine methyl ester on responses to allicin.** To investigate the role of nitric oxide in mediating allicin responses, the effects of N\textsubscript{\textsuperscript{ω}}-nitro-L-arginine methyl ester on pulmonary vasodilator responses to allicin were investigated under elevated tone conditions, and these data are shown in Fig. 3. Vasodilator responses were compared before and after administration of N\textsubscript{\textsuperscript{ω}}-nitro-L-arginine methyl ester (final reservoir concentration = 50 nmol/ml). Pulmo-

![Fig. 1. A: decreases in pulmonary arterial perfusion pressure in response to injections of 10- to 100-μg doses of allicin in the pulmonary vascular bed of rats. B: dose-response curves comparing decreases in pulmonary arterial perfusion pressure in response to adrenomedullin, calcitonin gene-related peptide (CGRP), and allicin.](http://jap.physiology.org/)

![Fig. 2.](http://jap.physiology.org/)

![Fig. 3.](http://jap.physiology.org/)
nary arterial pressure reached similar levels in both the control period and after treatment with N^ω-nitro-L-arginine with U-46619 (control, 36 ± 2 vs. 36 ± 3 mmHg, respectively). Responses to the vasodilators were compared before and beginning 10 min after administration of N^ω-nitro-L-arginine methyl ester. The decreases in pulmonary arterial perfusion pressure in response to allicin were not significantly reduced, whereas responses to acetylcholine (0.3 μg) were significantly reduced after administration of the nitric oxide synthase inhibitor (Fig. 3).

**Influence of sodium meclofenamate on responses to garlic compounds.** To investigate the role of cyclooxygenase and arachidonic acid metabolites in the mediation of responses to allicin, the effects of sodium meclofenamate on pulmonary vasodilator responses to allicin were studied and are represented in Fig. 4A. Vasodilator responses to allicin were compared when tone in the pulmonary vascular bed was elevated with U-46619 and with U-46619 + sodium meclofenamate. After administration of a 2.5 mg/kg body wt dose of sodium meclofenamate, decreases in pulmonary arterial perfusion pressure in response to allicin were not significantly different from the values obtained when allicin was injected during the control period. Sodium meclofenamate reduced the vasopressor responses seen in the pulmonary arterial perfusion pressure under low-tone conditions in response to injections of arachidonic acid (data not shown).

![Graph](image)

**Fig. 4.** A: influence of sodium meclofenamate on the decrease in pulmonary perfusion pressure in response to allicin under elevated-tone conditions. B: comparisons of responses to allicin and levromakalim before and after administration of the K_ATP channel antagonist U-37883A. *Significantly different from control.

**Influence of U-37883A on responses to garlic compounds.** To determine the role of K_ATP channel activation in mediation of the response to allicin, the effects of U-37883A on pulmonary arterial perfusion pressure response to allicin were investigated, shown in Fig. 4B. When pulmonary arterial pressure was increased by infusion of U-46619 in the presence of the K_ATP channel antagonist U-37883A, decreases in pulmonary arterial perfusion pressure in response to allicin were not significantly different from the values obtained when allicin was injected during the U-46619-only infusion period. However, decreases in pulmonary arterial perfusion pressure in response to levromakalim, a K_ATP channel agonist, were significantly reduced after U-37883A administration.

**Influence of ventilation on responses to garlic compounds.** The effects of ventilation on responses to allicin were also investigated. Obstruction of the main bronchus interrupts airflow to the lung and decreases lung volume as alveolar gas is absorbed. In these experiments, decreases in pulmonary perfusion pressure were compared when the lung was ventilated and when airflow to the lung was interrupted by main bronchus occlusion. The vasodilator responses to allicin were not different when the lung was ventilated and when airflow was interrupted (data not shown).

**DISCUSSION**

Results of the present investigation demonstrate that allicin induces dose-related decreases in pulmonary arterial perfusion pressure when tone in the pulmonary vascular bed is increased to a high-steady level with U-46619 in the rat. Furthermore, in experiments in which tone was raised to a high-steady level with a constant infusion of angiotensin II, allicin also induced dose-related decreases in pulmonary arterial perfusion pressure (unpublished observation, Kaye and De Witt). Inasmuch as pulmonary blood flow was maintained constant, the decreases in pulmonary arterial perfusion pressure reflect decreases in pulmonary vascular resistance. In terms of relative vasodilator activity in the pulmonary vascular bed, the dose of allicin required to decrease pulmonary arterial perfusion pressure by 3 mmHg was significantly smaller than the dose for calcitonin gene-related peptide to achieve the same effect, and little difference was seen between the doses of adrenomedullin and allicin required for the 3-mmHg decrease in pulmonary arterial perfusion pressure. The decreases in pulmonary arterial perfusion pressure were dose dependent and were not altered by administration of N^ω-nitro-L-arginine methyl ester, sodium meclofenamate, or U-37883A or by main bronchus occlusion. These results suggest that decreases in pulmonary vascular resistance in response to allicin appear to be independent of the release of endothelium-derived nitric oxide, activation of cyclooxygenase, mediation through K_ATP channels, or changes in bronchomotor tone.

These results also demonstrate that diallyl disulfide and allyl mercaptan did not significantly alter pulmo-
The release of nitric oxide. The observation that pulmonary vasodepressor responses to allicin were not affected by N\textsuperscript{\textast}nitro-L-arginine methyl ester, suggesting that allicin responses are independent of the release of nitric oxide. The observation that pulmonary vasodilatory responses to acetylcholine were decreased indicates that nitric oxide release was inhibited by N\textsuperscript{\textast}nitro-L-arginine methyl ester treatment. These results are in agreement with previous results in studies on the pulmonary vascular bed of the intact-chest cat. Furthermore, responses to allicin in that study were not sensitive to methylene blue, suggesting that the depressor response to allicin is independent of soluble guanylate cyclase in the pulmonary vascular bed of the cat. However, it has been recently suggested that the vasodilatory action of garlic in the rat is dependent on and involves the production of nitric oxide. The reason for the discrepancy in results is unknown but may be due to differences in the model used or duration of experimental protocols. However, because garlic plant chemistry is complex, the most likely reason for the difference is the impure nature and inconsistent garlic-type sulfur content used when dealing with administrations of garlic powder.

In the present study, pulmonary vasodilator responses to allicin were not altered by sodium meclofenamate in doses that blocked pressor responses to arachidonic acid, indicating that products in the cyclooxygenase pathway do not mediate or modulate responses. Moreover, allicin has been reported to produce a concentration-dependent inhibition of human platelet aggregation in vitro without affecting cyclooxygenase or thromboxane synthase activity or adenosine 3',5'-cyclic monophosphate levels. Currently, there are no known antagonists that inhibit the pulmonary or systemic vasodilator effects of allicin. The nature or type of allicin receptors in resistance vessel elements in the pulmonary vascular bed are unknown at present and require future studies.

In some organ systems, vasodilation has been reported to occur via hyperpolarization of vascular smooth muscle. Hyperpolarization of smooth muscle has been reported to occur through activation of an ATP-sensitive K\textsuperscript{\textast} channel. However, in the present studies, U-37883A did not alter responses to allicin in a dose that attenuated responses to the K\textsuperscript{\textast} channel opener levomakalim, suggesting that allicin does not dilate the pulmonary vascular bed of the rat by activating K\textsuperscript{\textast} channels. These results are compatible with previous studies demonstrating that garlic can induce vasodilation via smooth muscle cell membrane hyperpolarization and/or inhibition of the opening of calcium channels. These results suggest that, if hyperpolarization of smooth muscle cells does occur, it does not occur via the K\textsuperscript{\textast} channel.

It is unknown whether garlic derivatives have an effect on bronchomotor tone; however, it is possible that changes in bronchomotor tone may indirectly alter allicin-induced decreases in pulmonary perfusion pressure. Although there is no simple method of assessing the interaction between changes in airway and vascular smooth muscle tone in the lung, the effects of changes in bronchomotor tone can be minimized when airflow is interrupted, and the lung is in a low-volume state. The results of these studies demonstrate that decreases in pulmonary perfusion pressure in response to allicin are not changed during main bronchus occlusion. These data suggest that decreases in pulmonary perfusion pressure in response to allicin are independent of bronchomotor tone.

In conclusion, the results of the present study show that allicin has significant vasodilator activity in the pulmonary vascular bed of rat when tone is increased experimentally. However, diallyl disulfide and allyl mercaptan, metabolites of allicin, do not possess this vasodilatory action. Although the mechanism by which allicin induces vasodilatation is uncertain, the results of the present investigation suggest that this garlic derivative may be useful in the treatment of pulmonary hypertensive disorders, including primary pulmonary hypertension and chronic obstructive pulmonary disease. The present data also suggest that other allicin analogs should be developed for clinical use because of allicin’s vasodilator efficacy and apparent lack of toxicity.

REFERENCES


