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 allicin (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 allicin 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 meclofenamate, or when lung ventilation was interrupted. These data show that allicin 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 allicin 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.

allicin; allyl mercaptan; diallyl disulfide; lung circulation

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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 arteries.

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 38°C under conditions of elevated tone in the control period, the flow rate was set at 8–14 ml/min to raise pulmonary vascular tone to a level similar to that attained during the control period. In some experiments, 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.

N⁵-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 (Peptides International, Belmont, CA), calcitonin-gene related peptide (Bachem Bioscience, Philadelphia, PA), and U-37883A (Upjohn, Kalamazoo, MI) were dissolved in normal saline 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, stopped 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, N⁵-nitro-l-arginine methyl ester; group B, sodium meclofenamate; group C, U-37883A; and group D, man 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 N⁵-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 N⁵-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 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 Nω-nitro-L-arginine methyl ester on responses to allicin. To investigate the role of nitric oxide in mediating allicin responses, the effects of Nω-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 Nω-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. Compounds were injected into the perfused pulmonary artery, and baseline pressure in the perfused artery was raised to a high-steady value (35 ± 3 mmHg) with an infusion of U-46619. n, Number of experiments.

**Fig. 2.** Pulmonary arterial perfusion pressure changes in response to 0.3- and 1.0-mg injections of allyl mercaptan or diallyl disulfide into the pulmonary vascular bed of the rat.

**Fig. 3.** Comparisons of responses to allicin and acetylcholine before and after administration of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; final reservoir concentration = 50 nmol/ml) under elevated tone conditions. Compounds were injected directly into the pulmonary arterial perfusion circuit. *Significantly different from control.
nary arterial pressure reached similar levels in both the control period and after treatment with \(N^\omega\)-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 \(N^\omega\)-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 \(\mu\)g) 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).

**Influence of U-37883A on responses to garlic compounds.** To determine the role of \(K_{\text{ATP}}\) 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 \(K_{\text{ATP}}\) 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 \(K_{\text{ATP}}\) 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 \(N^\omega\)-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 \(K_{\text{ATP}}\) channels, or changes in bronchomotor tone.

These results also demonstrate that diallyl disulfide and allyl mercaptan did not significantly alter pulmo-
nary arterial perfusion pressure at a dose up to ten times the amount that produced vasodilation by allicin. Diallyl disulfide and allyl mercaptan are formed from the thiosulfinate allicin (17), and, indeed, the conversion of allicin to allyl mercaptan is quantitative in nature in vitro. It has been suggested that allyl mercaptan is the main active metabolite responsible for the proposed therapeutic benefits of garlic, including its antihyperlipidemic, antiatherosclerotic, anticancer, and antihypertensive actions (17). However, these results suggest that 1) the vasodilatory actions of garlic seen after acute administration are not dependent on diallyl disulfide and allyl mercaptan in the pulmonary vascular bed of the rat and 2) the thiosulfinate allicin or some other precursor is responsible for the vasodilatory action. It is unlikely that the allicin is responsible for the proposed long-term, systemic, antihypertensive effects observed after prolonged enteral intake of garlic, because the in vitro half-life of allicin, in general and in this preparation, is <1 min (12, 17). These results suggest that another component of garlic is responsible for these antihypertensive actions. One example may be the gamma-glutamylcysteines, found in aqueous-alcohol extracts of garlic and garlic powders, which inhibit the blood pressure-regulating, angiotensin-converting enzyme and might serve as the cause for the prolonged antihypertensive action of garlic (17).

Pulmonary vasodepressor responses to allicin were not affected by Nω-nitro-L-arginine methyl ester, suggesting that allicin responses are independent of the release of nitric oxide. The observation that pulmonary vasodilator responses to acetylcholine were decreased after acute administration are not dependent on diallyl disulfide and allyl mercaptan in the pulmonary vascular bed of the rat (7). 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


