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

ALAN D. KAYE,1 BRACKEN J. DE WITT,2 MUHAMMAD ANWAR,1 DONALD E. SMITH,2 CHANG J. FENG,2 PHILIP J. KADOWITZ,2 AND BOBBY D. NOSSAMAN2
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

Received 6 December 1999; accepted in final form 2 March 2000

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, or changes in bronchomotor tone in the pulmonary vascular bed of the rat.

allicin; 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 allicin. Allicin is an odorless, reactive interme-
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 (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 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. The flow rate was confirmed in some experiments by performing timed blood collection of pulmonary arterial pressure in response to sodium meclofenamate; group D, 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 N’-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 N’-nitro-l-arginine methyl ester (100 mg/kg ia) was attained, the U-46619 infusion was resumed as necessary 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 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 N\textsuperscript{ω}-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 cat (12). 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 (12). 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 (10). 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 (14). 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 (4, 8, 9). Hyperpolarization of smooth muscle has been reported to occur through activation of an ATP-sensitive K\textsuperscript{ATP} channel opener levcromakalim, suggesting that allicin does not dilate the pulmonary vascular bed of the rat by activating K\textsuperscript{ATP} 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. But these results suggest that, if hyperpolarization of smooth muscle cells does occur, it does not occur via the K\textsuperscript{ATP} 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 (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


