Effects of Amyl Nitrite on Pulmonary Vascular Resistance in the Dog* (32693)

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Amyl nitrite is a well-known and commonly employed musculotropic vasodilating agent. Its ability to decrease systemic vascular resistance has been repeatedly demonstrated in both animals and man (1–4). However, its specific action on the pulmonary circulation is not clearly defined.

Both increases and decreases in pulmonary artery pressure have been recorded following amyl nitrite inhalation (2,5–7). The variability of responses observed may reflect secondary changes that are obscuring the primary action of this agent on the pulmonary vasculature. Alterations in cardiac output and bronchial blood flow, changes in pulmonary blood volume and left atrial pressure, and possibly reflex responses could singly or in combination modify the expected response of the pulmonary vessels to the direct action of amyl nitrite.

In addition, pulmonary hemodynamic changes following administration of other nitrite or nitrate compounds have failed to yield conclusive results (2,8–12). Halmagyi *et al.* (8) have attempted to clarify seemingly contradictory results by emphasizing the importance of preexisting pulmonary vascular tone in determining the vascular response to these compounds.

In the present investigation the effects of amyl nitrite on pulmonary vascular resistance were studied in the isolated left lower lobe of the dog's lung. With controlled perfusion of the isolated lobe administration of amyl nitrite consistently resulted in a decline in pulmonary vascular resistance.

Methods. Ten mongrel dogs weighing between 11.3 and 17.7 kg were anesthetized with sodium pentobarbital (300 mg/kg) intravenously and placed on positive pressure respiration via endotracheal intubation.

A left thoractomy was performed in the fifth intercostal space. Heparin (5 mg/kg) was administered intravenously. The circulation to the left lower lobe of the lung was isolated from the remainder of the pulmonary circulation as follows. The lobar artery was dissected free of surrounding tissue and the proximal portion of the vessel was ligated. A polyethylene catheter, with a right-angle bend at the tip, was placed in the distal portion of the vessel and tied securely in place. In a similar manner venous effluent was collected by placing polyethylene catheters in the pulmonary vein or veins draining the isolated lobe. The bronchus was left intact to maintain adequate ventilation of the lobe.

The isolated lobe was perfused with autologous blood at controlled flow rates. The output of the Sigma-motor pump was adjusted to maintain mean pulmonary artery pressure between 10 and 20 mm Hg. All venous effluent was collected into an open-air reservoir and returned to the pump. The outlet of the pulmonary venous catheter was positioned slightly below the level of the pulmonary veins (—2 to —5 mm Hg) to insure adequate drainage. In this situation surrounding tissue pressure exceeds outflow pressure, but as pointed out by Permutt and Riley (13) in their description of vascular waterfall, this does not disturb flow through collapsible vessels.

In 6 of the 10 animals changes in reservoir level were continuously recorded by measuring alterations in the hydrostatic pressure of the blood in the reservoir. In all animals pulmonary artery, pulmonary venous, and systemic arterial pressures were simultaneously recorded via polyethylene catheters, pressure strain gages, and a direct-writing oscillograph. Airway pressure was not routinely recorded. However, previous experi-

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ments revealed no alterations of endotracheal pressure with administration of amyl nitrite.

Amyl nitrite was administered through the air inlet of the respirator until a change in systemic or pulmonary artery pressure was observed. In 6 of the 10 animals the administration of amyl nitrite was repeated after ventricular fibrillation was induced.

Results. Eighteen determinations of the effects of amyl nitrite on pulmonary vascular resistance were performed in 10 animals before ventricular fibrillation was induced. Alterations in pulmonary artery and pulmonary venous pressures refer to those recorded in the isolated left lower lobe. The systemic and the remainder of the pulmonary circulation were left intact and continued to function normally. Throughout each determination flow rate to the isolated lobe was held constant. In 17 cases there was a distinct fall in pulmonary artery pressure; in one instance there was no observable change. The average fall in pulmonary artery pressure was 11% of control levels. The time of onset of the decline in pulmonary artery pressure averaged 9 sec (range of 4–23 sec) after the initiation of amyl nitrite inhalation. Changes in pulmonary artery pressure preceded systemic arterial pressure changes in 12 experiments. In the remaining 6 experiments alterations in systemic arterial pressure occurred slightly ahead of or simultaneous with pulmonary artery pressure changes. In all cases pulmonary venous pressure remained unchanged.

Although systemic arterial pressure declined in every case, there was no definite relationship between the magnitude of the fall in systemic pressure and the change in pulmonary artery pressure observed in the isolated lobe. In Table I, the maximum changes in systemic

TABLE I. Effects of Amyl Nitrite on Pulmonary Artery Pressure in the Isolated Left Lower Lobe Before Ventricular Fibrillation Was Induced.

Animal No.	Control mean femoral artery pressure (mm Hg)	Mean femoral artery pressure following amyl nitrite inhalation (mm Hg)	% Change in mean femoral artery pressure	Control mean pulmonary artery pressure (mm Hg)	Mean pulmonary artery pressure following amyl nitrite inhalation (mm Hg)	% Change in mean pulmonary artery pressure
1	90	52	−42	12.0	10.5	−12
2	142	118	−17	13.5	13.0	− 4
	135	105	−22	14.0	13.5	− 4
3	83	60	−28	13.5	11.5	−15
4	87	52	−40	11.0	8.5	−23
	81	41	−49	11.0	8.5	−23
5	98	68	−31	13.5	12.5	− 7
	142	77	−46	12.0	12.0	—
	183	80	−56	12.0	11.0	− 8
6	140	65	−54	11.0	10.0	− 9
	131	91	−30	11.0	10.0	− 9
7	150	120	−20	14.0	12.0	−14
	143	106	−26	16.5	13.5	−18
8	107	37	−65	12.5	11.0	−12
	92	27	−71	14.0	12.0	−14
9	138	85	−38	11.0	10.0	− 9
	135	90	−33	12.5	11.5	− 8
10	115	105	− 9	15.0	14.0	− 7
Average values	127	79	−38	12.8	11.4	−11

arterial pressure and pulmonary artery pressure in the isolated lobe are listed for each experiment. The pulmonary artery pressure response to amyl nitrite was depressor and uniphasic and always exceeded the duration of systemic arterial hypotension (av: 243 vs 125 sec).

In 12 of the experiments venous effluent from the isolated lobe was recorded continously with the other parameters. A small, but detectable, increment of venous outflow over arterial inflow was observed during the control period. This disparity between venous outflow and arterial inflow was considered to represent a small contribution of blood from the bronchial circulation via communicating channels between the two vascular beds. Following amyl nitrite inhalation there was a noticeable increase in venous outflow as compared to arterial inflow (Table II).

To eliminate a possible reflex response in the circulation of the isolated lobe emanating from a sudden change in systemic arterial pressure, the studies were repeated in six of the animals after the induction of ventricular fibrillation. Systemic arterial pressure was allowed to remain at mean circulatory pressure. In this situation the alteration in pulmonary artery pressure in the isolated lobe following amyl nitrite inhalation was similar to that before ventricular fibrillation was induced (Fig. 1). The average percentage decline in mean pulmonary artery pressure was 16% (Table III). Reservoir level in this group of animals remained unchanged both during the control period and following amyl nitrite inhalation, indicating no contribution of blood from the bronchial circulation to the pulmonary venous effluent of the isolated lobe (Table II).

Discussion. The pulmonary vascular response to any vasoactive agent is difficult to assess in the intact animal. Changes in cardiac output and bronchial blood flow, changes in pulmonary blood volume, and possibly reflex responses, which are often associated with the administration of these agents, make it impossible to decide whether the changes observed are a primary or secondary effect.

Previous investigations have revealed both increases and decreases in pulmonary artery pressure following amyl nitrite inhalation (2, 5–7). In animal studies Love and McGuigan (2) postulated that whenever a rise in pulmonary artery pressure was observed, it was not produced by active constriction of the pul-

TABLE II. Effects of Amyl Nitrite on Bronchopulmonary Blood Flow in the Isolated Left Lower Lobe.

	Before ventricular fibrillation			After ventricular fibrillation	
Animal No.	Control bronchopulmonary blood flow (ml/min)	Bronchopulmonary blood flow after amyl nitrite (ml/min)	Change in bronchopulmonary blood flow (ml/min)	Control bronchopulmonary blood flow (ml/min)	Bronchopulmonary blood flow after amyl nitrite (ml/min)
5	0.0	4.0	+4.0	0	0
	4.0	7.0	+3.0		
	4.0	8.0	+4.0		
6	1.5	2.5	+1.0	0	0
	1.0	4.0	+3.0		
7	2.0	10.0	+8.0	0	0
	2.0	8.0	+6.0		
8	1.0	2.0	+1.0	0	0
	0.5	1.5	+1.0		
9	3.0	6.0	+3.0	0	0
	2.5	5.0	+2.5		
10	4.0	7.5	+3.5	0	0
Average values	2.1	5.5	+3.4	0	0

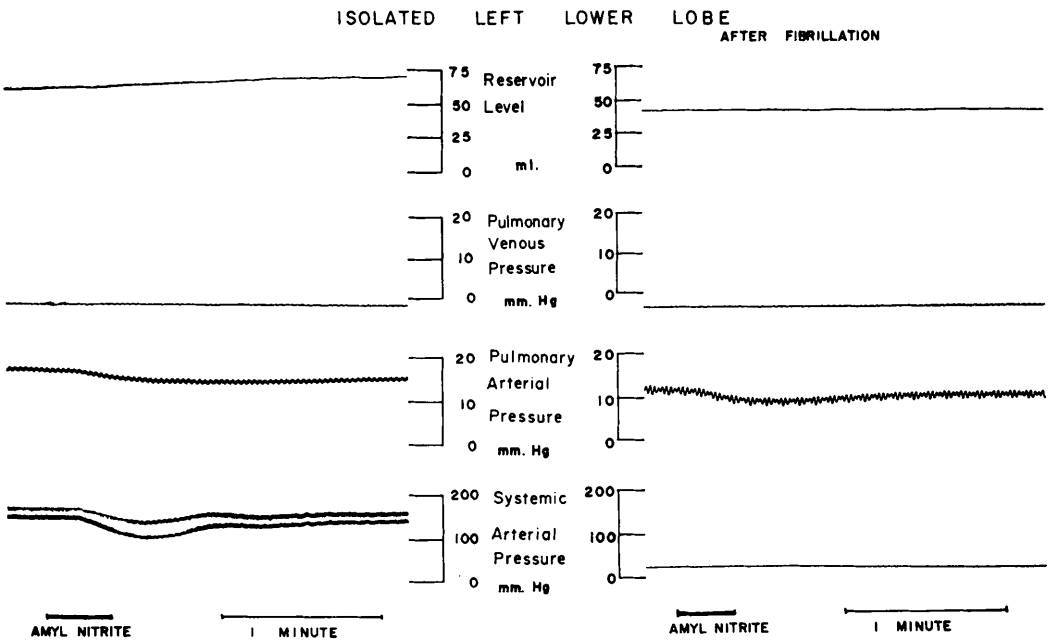

FIG. 1. Pulmonary vascular response to amyl nitrite in the isolated left lower lobe of the dog's lung. Amyl nitrite was administered both before and after ventricular fibrillation. Both tracings are taken from the same animal. A decline in pulmonary arterial pressure is evident in each case. Pulmonary venous pressure remains stable. The slight difference in the level of the pulmonary venous pressure in the two situations is due to an adjustment in the height of the pulmonary venous drainage catheter. Following ventricular fibrillation systemic arterial pressure remains constant at mean circulatory pressure. The gradual rise in reservoir level noted only when the systemic circulation is intact represents transfer of blood from the systemic to the pulmonary circulation via broncho-pulmonary anastomoses. Duration of amyl nitrite inhalation is indicated in the lower portion of each tracing.

monary vessels, but rather could be explained on the basis of a greater cardiac output resulting from cardiac acceleration and increased venous return. In a recent study in humans de Leon and Perloff (7) observed a small but significant rise in both systolic and mean pulmonary arterial pressures after the administration of amyl nitrite. In the sub-

TABLE III. Effects of Amyl Nitrite on Pulmonary Artery Pressure in the Isolated Left Lower Lobe Following Ventricular Fibrillation.

Animal No.	Control mean pulmonary artery pressure (mm Hg)	Mean pulmonary artery pressure following amyl nitrite inhalation (mm Hg)	% Change in mean pulmonary artery pressure
5	13.5	12.5	— 7
6	12.0	10.0	—17
7	11.0	8.5	—23
8	17.0	14.0	—18
9	16.0	14.0	—12
10	16.5	13.5	—18
	18.5	15.5	—16
Average values	14.9	12.6	—16

jects studied by de Leon and Perloff pulmonary blood flow increased out of proportion to the increase in pressure gradient indicating that a decline in pulmonary vascular resistance occurred. However, it was not possible to determine whether this reduction in pulmonary vascular resistance was passive in nature due to increased pulmonary blood flow or whether amyl nitrite exerted a direct vasodilating effect.

In the present series of experiments, the isolated lung lobe technique was utilized to make possible the direct observation of the action of amyl nitrite under controlled conditions. The data clearly indicate that amyl nitrite produces dilatation of the pulmonary vessels. In the presence of constant blood flow, the drop in pressure gradient across the pulmonary circulation can result only from a decrease in pulmonary vascular resistance. Furthermore, a reflex response emanating from a sudden decline in systemic pressure cannot be implicated since in the experiments in which ventricular fibrillation was induced and systemic pressure was allowed to remain constant, pulmonary vasodilation was still observed.

Collateral flow through bronchopulmonary anastomoses had little influence on the pulmonary vascular response to amyl nitrite. Previous studies have demonstrated that after ligation of the left pulmonary artery, collateral flow from the isolated left lower lobe of the dog varied from 4.4 to 9 ml/min. The magnitude of the bronchopulmonary flow varied with the level of the systemic arterial pressure (14). During amyl nitrite inhalation collateral flow was increased above control levels in the present experiments (Table II). This increase in collateral flow occurred in the presence of a sharp fall in systemic pressure suggesting that amyl nitrite may open additional communicating channels between the bronchial and pulmonary circulations. This allows greater transfer of blood from the bronchial to the pulmonary circulation. If this shift of blood volume were of sufficient magnitude to increase pulmonary artery pressure, the primary action of amyl nitrite on the pulmonary vessels would be modified. However, in the preparations in which systemic pressure and reservoir level remained constant, the pulmonary vascular response to amyl nitrite was still clearly evident. This demonstrates that the increased bronchopulmonary flow observed in the animals with an intact systemic circulation was insufficient to obscure the primary action of amyl nitrite.

These studies clearly show that the primary action of amyl nitrite on the pulmonary vasculature is vasodilatation. The rise in pulmonary artery pressure frequently observed following amyl nitrite inhalation in intact animals and man is probably not related to any reflex vasoconstriction of the pulmonary circulation or to an increase in bronchopulmonary flow, but rather is secondary to increased pulmonary blood flow.

Summary. The left lower lobe of the lung was isolated from the remainder of the pulmonary circulation and was perfused with autologous blood at controlled flow rates. All venous effluent from the lobe was collected into a reservoir. Pulmonary artery and pulmonary venous pressures, systemic arterial pressure and in some cases reservoir level were monitored continuously. In 17 out of 18 experiments in 10 dogs, amyl nitrite inhalation was followed by a decline (av 11%) in pulmonary artery pressure in the presence of constant blood flow. Pulmonary venous pressure was unchanged. Amyl nitrite inhalation was repeated in 6 of the animals after ventricular fibrillation was induced. In this situation systemic arterial pressure remained constant and thereby eliminated; (a) a possible pulmonary reflex response emanating from a sudden change in systemic arterial pressure and (b) an alteration in bronchopulmonary blood flow. The response of the pulmonary vasculature to amyl nitrite in this situation was similar to that before ventricular fibrillation was induced. These results indicate that amyl nitrite exerts a direct dilating effect on pulmonary vessels and decreases pulmonary vascular resistance.

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Lung Fibrinolytic Activity and Bovine High Mountain Disease* (32694)

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The etiology of high mountain disease of cattle (brisket disease) is still unknown though the pathological lesions have been studied in detail by many investigators (1). Characteristic is a medial muscular hypertrophy of the small pulmonary arteries and arterioles with a concomitant adventitial proliferation. There is also diffuse interstitial and alveolar septal fibrosis. Speculations on the etiology have centered upon the change in altitude and the lowered oxygen pressure, but these are not sole etiological factors since other domesticated animals (sheep, goat) do not develop the same disease pattern under identical conditions. An etiologic factor specifically related to cattle, but not to other animal species, has to be sought. Confusing the issue are the large individual variations in sensitivity to the disease (2,3).

Search for some property to discriminate the bovine lung from lungs of other species drew our attention to the presence of a potent inhibitor of fibrinolysis in the bovine lung. The bovine lung inhibitor (pulmin), first reported in 1950 (4,5), was later purified (6) and its effect on fibrinolysis studied in detail (7,8). Its probable identity with the inhibitor of Kallikrein and trypsin isolated from bovine parotid gland (Trasylol), and with the pancreatic trypsin inhibitor of Kunitz has recently been demonstrated (9,10).

Since brisket disease is characterized by proliferative processes, indicating a response to frequent tissue injury with release locally of tissue thromboplastin and plasminogen activator, it could be anticipated that the presence in certain tissues of a potent antifibrinolytic compound would affect the process of repair by delaying resolution of fibrin. Such considerations led to the suggestion that the trypsin inhibitor in bovine lung could be the etiological factor in brisket disease distinguishing cattle from other animal species (11). To substantiate this concept the fibrinolytic activity (tissue plasminogen activator concentration), trypsin inhibitor content, and thromboplastin concentration in lungs of various breeds of cattle were estimated.

Materials and Methods. Lungs were obtained fresh at slaughter houses and from agencies under the U.S. Department of Agriculture. Lungs from Brahman cattle were provided fresh by Dr. J. A. Boehm, then at Meat Inspection Division, Mid-State Meat Packers, Inc., Bartow, Florida. Samples were carefully rinsed under water, dried with absorbent paper, weighed and stored in tightly closed tubes at —20°C until investigated.

For *plasminogen activator* assays (12) tissue samples were homogenized with 3.0 ml of 2*M* KSCN per 100 mg tissue followed by slow mechanical stirring for 1 hour and centrifugation. An aliquot of the supernatant was diluted with 7 vol. of water, the pH was adjusted to 1 with N HCl, and the mixture

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