Effects of vasodilator drugs on normal and serotonin-constricted pulmonary vessels of the dog

ABRAHAM M. RUDOLPH, M. DAVID KURLAND, PETER A. M. AULD, AND MILTON H. PAUL (With the Technical Assistance of Sara R. Duncan and Mary Elizabeth Stone)
Department of Pediatrics, Harvard Medical School, and the Children’s Medical Center, Boston, Massachusetts

RUDOLPH, ABRAHAM M., M. DAVID KURLAND, PETER A. M. AULD AND MILTON H. PAUL. Effects of vasodilator drugs on normal and serotonin-constricted pulmonary vessels of the dog. Am. J. Physiol. 197(3): 617-623. 1959. Previous studies of the action of vasodilator drugs on the pulmonary blood vessels have yielded inconclusive results. Studies on the effects of continuous infusion of vasodilator agents, first on normal pulmonary vessels, and then on serotonin-constricted pulmonary vessels, suggest that the response of the pulmonary vasculature is dependent on the degree of vasoconstrictor tone present. Consistent vasodilator responses to acetylcholine, histamine and adenosinetriphosphate were elicited when the pulmonary vessels were constricted by continuous infusion of serotonin. Pulmonary vasomotor responses to drugs occur in both the small pulmonary vessels and the large pulmonary veins.

\[\text{Vasomotor activity in the pulmonary vasculature has been well demonstrated in several recent studies (1-3). Most of these studies have demonstrated vasoconstriction, but observations of vasodilator activity are limited. Several observers have demonstrated a mild reduction of pulmonary arterial pressure after intravenous injection of acetylcholine in patients with pulmonary arterial hypertension associated with mitral stenosis or congenital heart disease (4, 5). Fritts et al. (6) have also noted a small decrease in the mild pulmonary hypertensive response to hypoxia, after acetylcholine infusion. Although these investigations suggested a vasodilator response in pulmonary vessels, other reports have shown variable effects (3, 7). Normally the pulmonary vasculature is relatively dilated and the pulmonary pressure quite low. The effects of a dilator agent would, therefore, be difficult to demonstrate under these circumstances. In an attempt to demonstrate vasodilator response of the pulmonary vessels, the effects of various drugs on the normal pulmonary vessels, with normal pulmonary artery pressures, and then on the vessels continually constricted by 5-hydroxytryptamine infusion, with resultant elevation of pulmonary artery pressure, have been examined. The rapidly-acting drugs, acetylcholine, histamine and adenosinetriphosphate which have prominent systemic vasodilator properties, were chosen, and the effects of these agents were studied first on normal pulmonary vessels and then, under the same experimental conditions, on serotonin-constricted vessels. Simultaneous observations on the changes in systemic arterial vascular responses and on bronchial effects, were made. Evidence is presented to suggest that the response of the pulmonary vasculature to drugs depends on the state of vasomotor tone, and that previous administration of one drug may modify the response to other pharmacologic agents.}\]

\[\text{MATERIALS AND METHODS}\]

The studies were performed on 16 mongrel dogs weighing 12-16 kg. In 12 animals polyvinyl catheters were inserted into the pulmonary artery, aorta and left atrium through a left thoracotomy, to allow for repeated observations in the same animal (8). A period of at least 2 weeks following thoracotomy was allowed to elapse before any experimentation was conducted.

The individual experimental procedures were conducted as follows: the dogs were anesthetized by administration of sodium pentobarbital (30 mg/kg) into the pulmonary arterial catheter. The trachea was intubated with a polyvinyl tube with an inflatable cuff.
and positive pressure ventilation was maintained with a Palmcr respiration pump with a frequency of 16 per minute and a stroke volume of 200 ml. Several expired air collections in Douglas bags were made during the experimental procedure. The fraction of oxygen in expired air was determined from repeated measurements of partial pressure obtained by continuous withdrawal of expired air, beyond a mixing chamber, through a Beckman oxygen analyzer. Respiration was maintained with room air when the output was determined by the Fick method and with 100% oxygen when the dye-dilution method was used.

Simultaneous pulmonary arterial, aortic, left atrial and intratracheal pressures were measured by means of Statham P 23D pressure transducers and recorded on a Sanborn 4-channel direct-writing oscillograph. In some experiments intrapleural pressures were recorded by insertion of a water-filled polyvinyl tube into the pleural cavity via a needle. Cardiac output determinations were made repeatedly by either the Fick or dye-dilution method, T-1824 dye was used for the indicator. The dye injections were made into the pulmonary arterial catheter and the curve was inscribed by sampling from the aortic catheter through a Colson whole blood cuvette densitometer. Oxygen saturation of pulmonary arterial and aortic blood and oxygen capacity were determined by the spectrophotometric method (g).

A period of 20–30 minutes was allowed for stabilization of pressures and cardiac output. In view of the possible effects of drug administration on subsequent responses of pulmonary vasculature, two types of experiments were conducted. In the first, the effects of continuous infusion of the vasodilator drug alone were first observed, and after a period of recovery, the effects of the same infusion were examined during infusion of 5-hydroxytryptamine. In the second type of experiment, 5-hydroxytryptamine was infused and the vasodilator drug then administered during the 5-hydroxytryptaminic infusion. All drugs were administered with a constant infusion apparatus.

After preliminary trials, the doses used were: 5-hydroxytryptamine creatinine sulfate 75–100 μg/kg/min., acetylcholine 40–50 μg/kg/min., adenosinetriphosphate 75–100 μg/kg/min. and histamine diphosphate 1.0 μg/kg/min. These amounts were considered to be adequate to produce pulmonary vascular responses since they resulted in moderate to marked systemic vascular effects. The effects of 15 separate acetylcholine infusions alone, 3 infusions of adenosinetriphosphate alone, 4 infusions of histamine alone, and the results of 16 infusions of acetylcholine, 3 of adenosinetrithosphate and 4 of histamine during serotonin-induced constriction of the pulmonary vasculature were observed.

In two animals, acute experiments were performed. A left thoracotomy was performed under sodium pentobarbital anesthesia. A left pulmonary vein was catheterized by insertion of a 1.2 mm diameter catheter through the left atrium and advancing it 2.5–3 cm beyond the pulmonary venous and left atrial junction. Another catheter was advanced to the pulmonary artery wedge position. The experimental procedure was conducted as described in the chronic closed-chest preparation, but with the left thorax open, and measurement of pulmonary venous and pulmonary artery wedge pressures in addition to the other parameters.

In two other animals, the effects of rapid intravenous injection of small doses of acetylcholine, histamine diphosphate and 5-hydroxytryptamine, on aortic, pulmonary arterial and intratracheal pressures were studied. One hundred to one hundred and fifty micrograms of acetylcholine, 30 μg of histamine diphosphate and 500 μg of serotonin creatinine sulfate were administered separately, to observe the effects of the drugs individually. Acetylcholine and histamine diphosphate were then administered and as soon as the maximum effect was observed, serotonin was injected. Similar studies were performed after atropinization of the animal with 1 mg atropine/kg body weight.

**R E S U L T S**

The effects of acetylcholine, adenosinetriphosphate and histamine on pulmonary and systemic vascular re-
Acetylcholine

Pulmonary pressure. Average control pulmonary artery pressures were 22/10 mm Hg; mean pressures ranged from 8 to 18 mm Hg, with an average of 14.5 mm Hg. During acetylcholine infusion there was a consistent increase in pulmonary arterial systolic, diastolic and mean pressures. The rise in pressure started within a few seconds of the onset of infusion and reached a peak within 30-60 seconds. The pressure then gradually fell slightly, but was elevated above control levels as long as acetylcholine was administered. Repeated infusion of acetylcholine during the same experimental procedure resulted in almost identical responses of pulmonary artery pressure and no instance of tachyphylaxis was observed. The average rises of pulmonary arterial pressure were 6 mm systolic, 4 mm diastolic, and 5 mm Hg mean pressure, with a range of elevation of mean pressures of 1-11 mm Hg. Elevations of pressure persisted for periods up to 12-15 minutes as long as the infusion was continued, but pressure returned to control levels within 1-2 minutes after cessation of the infusion.

Systemic arterial pressure. The average control systemic arterial pressure was 120/94 mm Hg and mean pressure ranged from 85 to 170 with an average of 107 mm Hg. Pressures dropped rapidly following infusion of acetylcholine and became stable within 30-60 seconds. The average reduction was 26 mm Hg systolic, 22 mm Hg diastolic, and 25 mm Hg mean pressure, a decrease of 23% of control aortic mean pressures, to an average level of 103/72 with a mean of 82 mm Hg. The pressure reduction continued while acetylcholine was administered and returned to control levels within 3-5 minutes after cessation of the infusion. Repeated administration of the same dose of acetylcholine during the same experiment resulted in very similar response and no tachyphylaxis was noted.

Left atrial pressure. Average control left atrial mean pressure was 4 mm Hg and acetylcholine infusion resulted in only minor changes, causing either a small rise or small fall of mean pressure of 0.5-1 mm Hg.

Cardiac output. Average control cardiac output was 2.4 l/min. with a range of 1.6-3.0 l/min. During acetylcholine infusion there was a consistent increase of cardiac output to an average level of 3.1 l/min. with a range of 2.6-4.2 l/min. (a rise of 29% above control levels). After administration of acetylcholine was stopped cardiac output returned to near control values within 3-5 minutes.

Heart rate. A consistent increase in heart rate was produced during the acetylcholine infusion. The increased rate coincided with the fall in systemic pressure, averaged 12% of control level, and persisted as long as the systemic pressure was reduced.

Intratracheal and intrapleural pressures. There were no consistent or significant changes in intratracheal or intrapleural pressures during acetylcholine infusion. In some instances a rise of 1-2 cm of water in positive phase of intrapleural pressure was observed.

Pulmonary and systemic vascular resistance. The average control pulmonary vascular resistance was 4.4 mm Hg/l/min. No change in calculated resistance occurred in four instances. A small decrease of 0.6-1.1 mm Hg/l/min. which should be regarded as of questionable significance in view of difficulties in determination of pulmonary vascular resistance, occurred in 7 infusions; in the remaining four cases, there was a small increase of pulmonary resistance of 0.4-1.0 mm Hg/l/min. Systemic vascular resistance dropped markedly in all instances from an average control level of 41.8-15 mm Hg/l/min., confirming an adequate vasodilator response during infusion of the drug.
stopped.
an average level of continuing for the period of infusion of the added drug, and returning to the previous level when the infusion was attained reduction of pressure was now produced, con-
tained by the vasodilator drugs alone, a consistent and sus-
tainable reduction with acetylcholine when pulmonary vascular re-
sistance was raised by serotonin infusion. Time scale 10 min.

**Adenosinetriphosphate (Dipotassium Salt)**

The responses to infusion of this substance were similar to those with acetylcholine infusion—a 12% average rise in pulmonary arterial mean pressure, a 14% decrease in systemic arterial mean pressure, a 15% rise in cardiac output, with no significant changes in pulmonary vascular resistance or intratracheal pressure.

**Histamine**

Histamine infusion produced similar responses with a 24% rise in pulmonary arterial mean pressure, a 34% decrease in systemic arterial mean pressure, a 26% rise in cardiac output, and minimal increase of pulmonary vascular resistance, with no change of intratracheal pressure.

**Acetylcholine, Adenosinetriphosphate and Histamine Effects During 5-Hydroxytryptamine Infusion**

The responses to these vasodilator drugs during 5-hydroxytryptamine infusion were essentially similar in type, but not in degree (table 1, figs. 1b, 2).

**Pulmonary arterial pressure.** The average pulmonary arterial pressure during continuous 5-hydroxytryptamine infusion was 48/22 mm Hg with a mean pressure average of 32 mm and a range of 20-43 mm Hg. In contrast to the increase of pulmonary arterial pressure induced by the vasodilator drugs alone, a consistent and sustained reduction of pressure was now produced, continuing for the period of infusion of the added drug, and returning to the previous level when the infusion was stopped.

Acetylcholine reduced pulmonary arterial pressure to an average level of 41/20 mm Hg with an average mean pressure of 27 mm Hg and a range from 14-35 mm Hg. T...
Pulmonary Artery Wedge, Pulmonary Venous and Left Atrial Pressure Relations

In the two open-chest preparations in which these relations were measured, the effects of acetylcholine alone, histamine alone, 5-hydroxytryptamine, and acetylcholine and histamine during 5-hydroxytryptamine infusions, were examined.

With acetylcholine and histamine alone, the mild increase of pulmonary arterial pressure was associated with an increase of both pulmonary artery wedge, and pulmonary venous pressure, with no significant change in left atrial pressure (fig. 3). During repeated infusion of acetylcholine and histamine, elevation of pulmonary arterial mean pressure was 4-6 mm Hg, and of pulmonary venous pressure, 2-3 mm Hg.

5-Hydroxytryptamine infusion produced elevations of pulmonary arterial mean pressure of 14 and 18 mm Hg, increase of pulmonary arterial wedge pressure of 7 and 11 mm Hg, and of pulmonary venous pressure of 2 and 3 mm Hg. Acetylcholine and histamine infusion during continuous 5-hydroxytryptamine administration resulted in decreases in pulmonary arterial pressure of 3-6 mm, in pulmonary arterial wedge pressure of 2-5 mm, and in pulmonary venous pressure of 2-3 mm.

Effects of Rapid Injection of Acetylcholine, Histamine and Serotonin

Small doses of acetylcholine, histamine and serotonin injected rapidly into the venous system resulted in a rise of pulmonary arterial pressure and a short-lived rise in intratracheal pressure. The effects of serotonin were proportionately much greater than those of the other drugs.

If acetylcholine or histamine were injected, and serotonin administered when the maximum systemic vascular effect of these drugs had occurred, pulmonary arterial response to serotonin was considerably decreased. Both height and duration of pulmonary arterial pressure response to serotonin were decreased as compared to the effect of serotonin alone, whereas airway pressure response was essentially the same (fig. 4).

Atropinization of the animal completely prevented the usual responses to acetylcholine injection, and also inhibited the effect of previous acetylcholine injection on serotonin response. The effects of histamine and serotonin were, however, similar to those prior to atropinization (fig. 5).

Discussion

Vasoconstriction in the pulmonary vascular system has been conclusively demonstrated in recent studies (1). The effects of vasodilator agents have, however, been extremely variable, and reports in the literature have shown great inconsistency in response of the pulmonary vessels to the same drug. Most difficult to reconcile has been, however, the fact that in the majority of animal experiments, acetylcholine, histamine, aminophylline and adenosinetriphosphate have produced minor but fairly consistent vasoconstriction of the pulmonary vessels (3, 7, 10, 11); whereas administration of those drugs to patients has in many instances caused a reduction in pulmonary vascular resistance (4, 5, 12). The most prominent vasodilator effects in human beings have been observed in patients with moderate pulmonary arterial hypertension and Harris (5) has suggested that the variability in responses in patients may depend on the degree of tone in the small pulmonary arteries.

Changes in pulmonary vascular resistance should be interpreted with caution, since they may be affected by changes in left atrial pressure, pulmonary blood volume, intrapleural and intratracheal pressures, cardiac output and blood viscosity, as well as reflect changes in vasomotor tone in response to a test agent. Most of these factors were continuously monitored in the experiments described.

The minimal changes observed in our studies with the vasodilator drugs alone, are possibly not significant. The consistent rise in pulmonary arterial pressure could possibly be explained on the increase in cardiac output, and tachycardia, which are probably related to a reflex response to systemic hypotension. However, a rise of cardiac output of this degree does not usually produce an increased pulmonary arterial pressure in a normal pulmonary vascular system and thus in spite of the minimal changes in pulmonary vascular resistance, it is suggested that an increased tone is produced by action of these drugs on normal pulmonary vessels.

This probable vasoconstrictor response to acetylcholine is in agreement with the observations of Rose (7) and Borst et al. (3). Observations in a perfusion preparation in which all the above-mentioned factors which may affect pulmonary vascular resistance are maintained constant, confirm the vasoconstrictor effect of acetylcholine and histamine in normal pulmonary vessels (unpublished observations).
Administration of the vasodilator drugs when the pulmonary vessels were constricted by 5-hydroxytryptamine showed consistent vasodilation. There were no changes in cardiac output, left atrial pressure, heart rate or intrathoracal pressure or intrapleural pressures, but calculated pulmonary vascular resistance decreased significantly in all instances. This observation has also been confirmed in the perfusion preparation (unpublished observations). These findings concur with those of Harris (5), Wood et al. (4) and Fritts et al. (6).

The action of acetylcholine, histamine and adenosine-triphosphate on the pulmonary vessels therefore appears to depend on the degree of vasoconstriction tone present. The normal peripheral vessels are relaxed and show either no change or a mild increase in vascular tone. In constricted vessels, whether the increased tone is due to 5-hydroxytryptamine, anoxia, or disease, a consistent vasodilator response may be elicited.

The possibility that acetylcholine, histamine and adenosine-triphosphate are serotonin inhibitor drugs certainly has to be considered. These substances did not, however, have any effect on the bronchoconstriction produced by 5-hydroxytryptamine whereas all the serotonin-inhibitors tested consistently reduced airway pressure (7) as well as pulmonary arterial pressure. Furthermore, in the presence of a very high infusion rate of 5-hydroxytryptamine, the same decrease in pulmonary arterial pressure and pulmonary vascular resistance, resulted, without any effect on airway pressure. These observations suggested that the effects were not related to any specific antiserotonin effect. Atropinization prevented the effects of acute acetylcholine injection on serotonin response, but did not prevent the histamine effect. This adds further support to the fact that acetylcholine is not acting as a chemical serotonin inhibitor.

The drugs examined appear to affect the large pulmonary veins as well as the small pulmonary vessels. Demonstration of an increased gradient between pulmonary artery wedge and pulmonary venous pressure with 5-hydroxytryptamine, acetylcholine and histamine infusion indicates that some of the vasoconstriction occurs in the small pulmonary vessels (arterioles, capillaries or venules). Development of an increased pressure gradient between the large pulmonary vein and left atrium suggests that there is vasomotor activity in the large pulmonary veins near their entrance into the left atrium. Pulmonary venous vasomotor activity has been demonstrated by Franklin in isolated vessel segments and suggested by Rivera-Estrada et al. (14) as the cause of anoxic pulmonary hypertension. Gilbert and co-workers (15) have also demonstrated pulmonary venous reactivity to drugs in an isolated perfused lung preparation. Our findings suggest that 5-hydroxytryptamine appears to exert its effects mainly on the small pulmonary vessels, with a relatively mild pulmonary venous effect, whereas acetylcholine and histamine affect predominantly pulmonary veins.

In evaluating these results, it should be appreciated that pulmonary venous to left atrial pressure gradients should be interpreted with caution, since they may be markedly affected by catheter size, and changes in pulmonary blood flow. The above-mentioned findings, however, have been confirmed in constant pulmonary flow experiments (unpublished observations).

The drugs infused exert a very rapid effect in the pulmonary vascular system and appear to act locally directly on the vessels. If the infusion is made into the left atrium, the effect on the pulmonary vasculature is delayed for several seconds, as is the effect on the bronchi. The observations of pulmonary vascular responses to drugs thus confirm that these vessels are capable of vasoconstrictor and vasodilator responses. In evaluating pulmonary vascular responses to pharmacologically active agents, it should be appreciated that previous administration of one drug may significantly modify the response to other drugs. The validity of many previous observations on the effect of drugs on the pulmonary vasculature is thus open to question since
numerous agents were tested without assuring adequate recovery. The normal pulmonary vasculature is relatively dilated and previous studies of the action of vasodilator drugs on these vessels have yielded inconclusive results. The effects of continuous infusion of acetylcholine, histamine and adenosinetriphosphate on normal pulmonary vessels, and then on vessels continually constricted by 5-hydroxytryptamine infusion have been examined.

Acetylcholine produced a consistent mild increase of pulmonary arterial pressure, a rise in cardiac output and minor and inconsistent changes in pulmonary vascular resistance in the normal dog; but during serotonin infusion, acetylcholine resulted in a consistent decrease of pulmonary arterial pressure and pulmonary vascular resistance. Similar effects were produced by histamine and adenosinetriphosphate. Evidence is presented that these effects were not due to serotonin inhibition. These findings suggest that the response of the pulmonary vasculature may depend on the degree of vasoconstrictor tone present.

Pulmonary vasomotor activity in these experiments has been demonstrated to occur in both the small pulmonary vessels as well as the large pulmonary veins. Serotonin exerts its major effect on the small pulmonary vessels, whereas acetylcholine and histamine affect predominantly pulmonary veins.

REFERENCES