Active Constriction and Dilatation in Pulmonary Circulation in Response to Acetylcholine.* (23069)

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Many believe that the pulmonary circulation is passive, in that changes in vessel calibre are due only to changes in blood flow or alterations in intrapulmonary blood volume. The observed effects of drugs on pulmonary arterial pressure have been attributed to back-pressure from the left auricle(1), redistribution of blood volume(2) or altered bronchomotor tone(3). However, recent reports (4,5,6) of experiments that excluded extrapulmonary factors indicate that vessels of the lesser circulation are capable of active vasoconstriction in response to appropriate pharmacologic stimuli. Older experiments showed that acetylcholine produces vasomotor responses in isolated pulmonary vessel segments (7) and perfused resected lungs(8).

The present report is concerned with the use of this drug in demonstrating ability of lesser circulation to actively dilate, and in investigating relationships between pulmonary vasomotor and bronchomotor activity.

Methods. A diaphragm type of pump was substituted for the left ventricle in dogs. The apparatus and technic for establishment of this extracorporeal circulation have been described in detail(9). Blood was drained totally from left auricle via a 3/8 I. D. Tygon tube to a reservoir. From the reservoir it was pumped to a T-tube in the descending thoracic aorta. The left ventricle thus was by-passed. The right ventricle continued to function effectively, keeping pace with the mechanical pump. Stroke volume and rate of "left ventricular" pump were kept constant at levels between 1.8 and 2.4 l minute. Pump output does not vary with alterations in peripheral vascular resistance(9). The modification by which effects of drugs in the pulmonary circulation were studied, has also been described(6). As the drug under study was injected into the main pulmonary artery, the tube draining the left auricle was diverted to a second reservoir. The systemic circulation continued to be maintained with blood from main pump reservoir. When main pump reservoir was near depletion, the left auricular tube was returned to its original position. Then, after an interval, the blood and drug in the temporary container were returned to the system. During period in which the left auricular tube was diverted from main pump reservoir, the drug traversed only the lungs and was excluded from the systemic circulation and ventricles. All studies were controlled with saline injections of comparable volumes. Acetylcholine was injected in volumes never greater than 1 ml. In several instances, instead of direct pulmonary arterial injection, the drug was introduced through a polyethylene catheter, the tip of which was placed in the right ventricle. This was to obviate any "streamlining" effect with unequal distribution to various portions of the lungs. No qualitative or quantitative differences could be noted between intraventricular and direct pulmonary arterial injection. Mongrel dogs weighing 12 to 18.5 kg were anesthetized with sodium pentobarbital 25 mg/kg. All studies were made in open-chest animals. The trachea was intubated and respirations were maintained with 100% oxygen delivered via variable phase pulmotor valve. In nebulization experiments, acetylcholine was delivered into airway in 1% concentrations with Vaponephrin® nebulizer under pressure, through 4 or 5 respirations. Experiments were controlled with nebulizations of normal saline. Cervical vagotomies performed in 3 experiments were bilateral. Methods for pressure and flow recording have been previously described(9). In the present experi-

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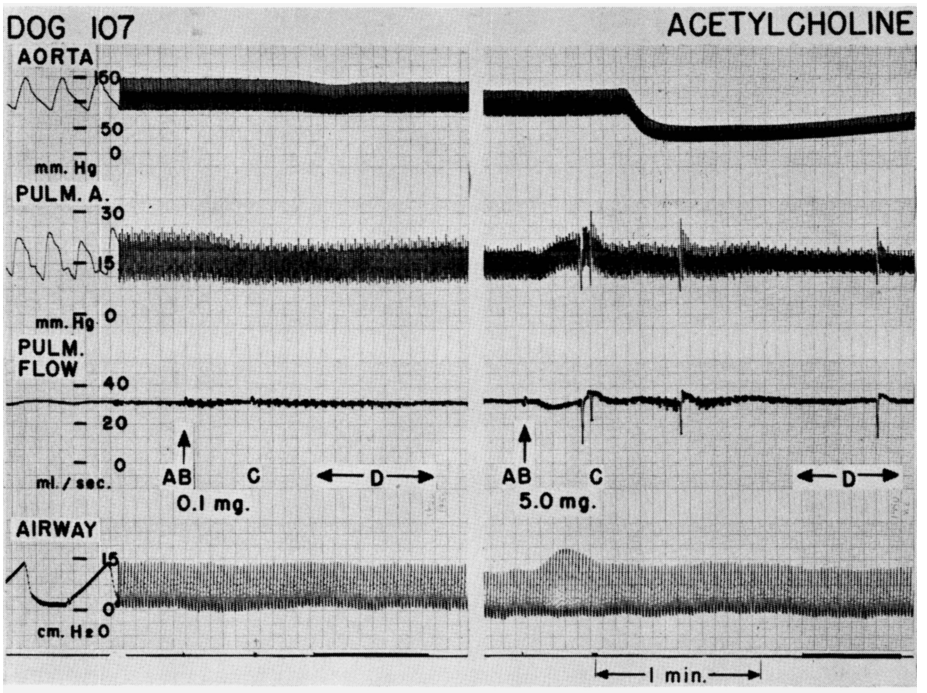

FIG. 1. Original records of injection of 8 μg/kg (left) and 400 μg/kg (right) acetylcholine into main pulmonary artery. At (A), left auricular tube was diverted to temporary reservoir. At (B), injection was made. At (C), left auricular tube was returned to its original position. Between (B) and (C), the drug traversed only the lungs. At (D), blood in the temporary reservoir, which contained the drug that had traversed the lungs, was returned to the experimental system. The smaller dose produced a fall in pulmonary arterial pressure and a slight increase in flow with no change in airway pressure. The larger dose caused a rise in pulmonary arterial pressure and a fall in flow, with an elevation of airway pressure. The fall in aortic pressure following (C) on right occurred before the drug in temporary reservoir was returned to circulation and was probably due to small amounts of acetylcholine still washing out of the lungs.

ments, left auricular blood flow was monitored with a Shipley-Wilson rotameter.

Results. Acute effects of acetylcholine injected into pulmonary artery. Preliminary screening of many drugs in this experimental preparation revealed that acetylcholine was a particularly potent agent for increasing pulmonary vascular resistance. However, since these observations were made only on vascular pressures and flow, and since Rodbard(3) has suggested that the acetylcholine response is due to altered bronchomotor tone, these experiments were repeated with continuous monitoring of intratracheal (airway) pressure. The following data are taken from observation of 31 injections of acetylcholine in 10 experimental preparations.

In doses of 0.1 to 5 mg (9 to 400 μg/kg) acetylcholine produced increased pulmonary vascular resistance (6 to 150% of control value) in 13 experiments. An example of this response is shown in the record on the right side of Fig. 1. In each instance, the increased pulmonary vascular resistance was accompanied by acute rise in airway pressure of variable magnitude.

On the left side of Fig. 1, the record is representative of the response noted in 18 experiments in which 0.01 to 0.15 mg (0.5 to 10 μg/kg) acetylcholine were injected into the pulmonary artery. In these instances, little or no rise in airway pressure was detectable, and pulmonary vascular resistance fell 6 to 29% of control value.

In neither of the above groups was it possible to establish a dose-response relationship. However, in individual experimental preparations, it was possible to clearly draw the line at which the pulmonary depressor response changed to a pressor response. The minimal

dose at which a pressor response was noted ranged between 9 and 70 μg/kg acetylcholine injected in the pulmonary artery. A point at which no resistance alteration resulted because of balancing of opposing effects was never detected. Varying experimental conditions and gradual development of tachyphylaxis, especially to smaller doses, may have contributed to the inability to quantitate these effects any further.

Response to acetylcholine following bilateral cervical vagotomy. Respiratory and vascular reflexes mediated by the vagi have been demonstrated as arising from pulmonary blood vessels in response to a variety of chemical(10) and mechanical(11) stimuli. These have been demonstrated with the present experimental preparation(12). Following bilateral cervical vagotomy in 3 dogs, the pulmonary vasopressor and bronchomotor responses to acetylcholine (60 to 200 μg/kg) injected into the pulmonary artery were unaltered. This indicates that the observed vascular and bronchial responses probably were directly elicited by the drug rather than by reflex stimulation.

Reexamination of effects of pressor amines with respect to bronchomotor activity. Increased pulmonary vascular resistance in response to epinephrine and norepinephrine was demonstrated by the same technic(6). However, airway pressure was not recorded. In 3 dogs, norepinephrine was retested in doses of 6 to 12 μg/kg injected into the pulmonary artery. In no instance was there an increase in intratracheal pressure. In one case, there was a fall in airway pressure simultaneous with increased pulmonary vascular resistance.

Effects of nebulized acetylcholine on pulmonary vascular resistance. Introduction of 1% acetylcholine into the airway via the nebulizer produced an average rise in maximum inspiratory airway pressure of 39% (range 15 to 80%) in 6 experiments in 5 preparations. The maximal rise occurred between 25 and 110 seconds following nebulization. There was no rise in pulmonary arterial pressure or alteration in pulmonary venous flow in any instance at time of maximal airway pressure increase.

Discussion. The means by which acetylcholine injected in the pulmonary artery affects the bronchi remains obscure. Marchand, Gilroy and Wilson(13) demonstrated anastomoses between the pulmonary and bronchial circulations in humans, and these have been discussed in relation to the dog pulmonary circulation by Daly *et al.*(14). It is possible that acetylcholine enters the bronchial capillary or venous circulation via such pulmonary-bronchial anastomoses, so that bronchomotor effects could occur even when the pulmonary circulation is isolated from the systemic circulation. On the other hand, it is possible that respiratory units provided with sufficient smooth muscle to produce significant elevations of intratracheal pressure are supplied by the pulmonary artery.

The relationships between bronchomotor and pulmonary vasomotor activity remain to be further clarified. It remains to be determined whether intratracheal pressure accurately reflects pressure throughout all portions of the bronchial tree.

The qualitatively different pulmonary vessel response to large and small doses of acetylcholine was demonstrated by Gaddum and Holtz in perfused lungs(8). In the present studies in which normal pressures, flows, and ventilation were maintained, the older findings were confirmed. The recent investigation of Harris and his colleagues(15) suggests that acetylcholine produces vasodilatation in the human pulmonary circulation and that this effect is augmented by preexisting increased tone in the pulmonary vessels. None of the investigations cited suggest which are the most active segments in the lesser circulation.

Summary and conclusions. The effects of acetylcholine on pulmonary vascular bed of intact dogs were studied in an experiment in which the drug could be excluded from the systemic circulation. While larger doses (usually greater than 0.1 mg) injected into the pulmonary artery were followed by increased pulmonary vascular resistance, smaller doses lowered pulmonary vascular resistance. Exclusion of extrapulmonary factors indicated active pulmonary vasoconstric-

tion and vasodilatation. The larger doses also caused bronchomotor activity. However, the lack of intratracheal pressure changes with pulmonary vasodilating doses of acetylcholine or vasoconstricting doses of norepinephrine, and the absence of pulmonary hypertension with nebulization of acetylcholine indicated that pulmonary vascular resistance alterations were not dependent upon changes in bronchomotor tone.

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Molybdenum in Poult Nutrition.* (23070)

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Molybdenum has been reported to be associated with xanthine oxidase in the rat(1, 2). The maximum level of molybdenum required for optimum xanthine oxidase activity was estimated by De Renzo(3) to be 0.2-0.3 μg/day; however, rate of growth of weanling rats was unaffected by molybdenum supplementation. It was reported by Reid *et al.* (4) that addition of molybdenum to purified diets for chicks or poults resulted in stimulation of growth rate similar to that obtained with distillers dried solubles ash. Remy and Westerfeld(5) were unable to demonstrate an effect of liver residue on xanthine dehydrogenase in the chick as had been obtained in the rat. Higgins *et al.* obtained molybdenum deficiency through addition of 4.5-9.4 mg% tungsten in the ration of chicks. Feeding of molybdenum restored xanthine dehydro-

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