Effects of Hypoxia on Pulmonary Vascular Reactivity in Pneumonectomized Puppies and Minipigs

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ABSTRACT

Pneumonectomy was performed on 25 1-month old beagle puppies and 13 2-month old minipigs. Three weeks or more later, pulmonary blood flow and pulmonary artery pressure were measured in the survivors and total pulmonary vascular resistance was calculated in each animal. These measurements were made at normal arterial Po2 and at an arterial Po2 of 31 ± 5 mm Hg produced by ventilating the animals with 10% oxygen in nitrogen. Seven normal puppies and seven normal minipigs of ages comparable to the pneumonectomized animals were studied as controls. The mean pulmonary artery pressure in the pneumonectomized puppies was not significantly different from that of the normal puppies. There was no significant pulmonary vascular response to hypoxia in the pneumonectomized puppies. The pneumonectomized minipigs had a significantly higher resting mean pulmonary artery pressure than the normals (P < 0.005). They did not, however, show a greater reactivity to hypoxia than did their normal litter mates. Both pneumonectomized and normal minipigs were more reactive to hypoxia than puppies. The minipigs were restudied approximately 16 weeks after the initial study. Four out of five animals showed a further rise in resting pulmonary artery pressure. Histological studies showed arteriolar medial hypertrophy in the minipig lungs.

KEY WORDS
pulmonary vascular resistance
medial hypertrophy
pulmonary artery pressure
pulmonary vascular disease
arterial blood oxygen tension

How does increased pulmonary blood flow cause progressive pulmonary vascular disease? This question has been under investigation for many years. Possible influences on increased flow such as systemic hypoxia, increased pulmonary artery pressure, left atrial hypertension, and pulmonary artery alkalosis have also been studied. Lack of a satisfactory experimental model in which to study these problems has added to the inherent difficulties.

Pulmonary vascular changes can be induced by the surgical creation of aortic-pulmonary shunts (1, 2). This technique not only increases pulmonary blood flow but elevates pulmonary artery pressure and pulmonary venous pressure. The effects of flow alone can be studied after pneumonectomy or ligation of one pulmonary artery which deflects the entire cardiac output through the remaining lung. Rudolph et al. used these techniques to produce a significant increase in pulmonary artery pressure in a small group of puppies and adult dogs (3). In chronic hypoxia induced in calves by an altitude of 5280 feet, Vogel et al. (4) produced a reactive pulmonary circulation by unilateral pulmonary artery ligation.

Seeking an experimental model of the pulmonary circulation which would react briskly to hypoxia and other stimuli we have reexamined the effects of pneumonectomy in puppies and minipigs.

Method

Twenty-five pure-bred beagle puppies and 13 minipigs (Improved Pitman Moore strain) under-
went pneumonectomy. All puppies operated on before 4 weeks of age died as did all puppies subjected to right pneumonectomy. Thirteen puppies survived left pneumonectomy done between 4 and 8 weeks of age. The 13 minipigs underwent left pneumonectomy between 11 and 17 weeks of age. There were 8 survivors.

Hemodynamic studies of the 13 surviving puppies were done not less than 3 weeks after pneumonectomy. Light general anesthesia was induced with pentobarbital (20 mg/kg iv). The puppies were intubated and ventilated with room air with a Harvard small animal respirator adjusted to maintain normal blood pH and gases. Cardiac catheters were advanced to the ascending aorta and main pulmonary artery, and pressures were recorded using Statham P23D transducers and an Electronics for Medicine recorder. Cardiac output was measured by indocyanine green and a Waters X300 densitometer. The cardiac output was obtained from the curve by using a nomogram (5). Arterial blood gases were measured with an Instrumentation Laboratory meter.

Once base-line measurements of flow, pressure, and blood gases were stable, the ventilating gas was altered to 10% oxygen in nitrogen and maintained at this level for 5 minutes. Pressure and flow measurements were then repeated. The animals then breathed room air, and when pulmonary artery pressure and pulmonary blood flow had returned to prehypoxic levels, three animals were subjected to an infusion of 0.3M lactic acid. The effect on pulmonary artery pressure and blood flow was again measured. Ventilation was maintained at a constant tidal volume and rate during these procedures.

The minipigs that survived pneumonectomy were studied 6 to 9 weeks after surgery. Anesthesia was induced with oxygen, nitrous oxide and 1.5% methoxyflurane. The animals were intubated and ventilated with a Palmer pump adjusted to maintain normal blood pH and gases. Anesthesia was maintained with minimum effective doses of pentobarbital. The experimental protocol from there on was similar to that used for the puppies. Five minipigs were restudied 16 to 19 weeks after pneumonectomy. Finally, eight normal minipigs, litter mates of the pneumonectomized animals, were studied to provide control data.

**Results**

All results are given as means ± 1 sd.

The pneumonectomized beagle puppies had a resting pulmonary artery mean pressure of 15.5 ± 5.5 mm Hg compared with 14 ± 2.5 in a group of seven normal puppies with comparable pulmonary blood flows (Fig. 1).
Cardiac output of 1.2 ± 0.3 liter/min in the pneumonectomized puppies compared with 1.1 ± 0.2 liter/min in the controls. Neither severe hypoxia nor lactic acid infusion produced a significant increase in pulmonary artery pressure. The mean pulmonary artery pressure prior to hypoxia (P02 75 ± 5) was 15 ± 6 mm Hg and during hypoxia (P02 31 ± 7) it was 16.5 ± 4 mm Hg (Fig. 2). Prior to lactate infusion mean pulmonary artery pressure was 14 ± 6 and after infusion 15.5 ± 7. Serial studies in four pneumonectomized puppies showed no tendency for pulmonary artery pressure to rise progressively (Fig. 3).

The pneumonectomized minipigs had a significantly higher resting mean pulmonary artery pressure than normal minipigs of the same age (31 ± 3.5 mm Hg vs. 19 ± 2.7 mm Hg, P < 0.005) (Fig. 1). Cardiac output was 2.7 ± 0.4 liter/min in the pneumonectomized animals and 2.5 ± 0.7 liter/min in the control animals. The pneumonectomized minipigs did not show a greater reactivity to hypoxia than their normal litter mates. Both pneumonectomized and normal minipigs, however, were more reactive than puppies (Fig. 2).

Four out of five minipigs that were restudied showed a further increase in mean pulmonary artery pressure. In the remaining animal there was no change (Fig. 4). Histological studies on lung sections from the pneumonectomized minipigs showed medial hypertrophy in the pulmonary arterioles. No intimal lesions were seen (Fig. 5).

Discussion

Before birth, the pulmonary artery pressure equals that in the descending aorta, and its precise level is determined by the cardiac output and the combined vascular resistances of the systemic, pulmonary, and placental circulations. Once the ductus arteriosus constricts, pulmonary artery pressure depends on right ventricular output and pulmonary vascular resistance only. Normally, in man and other species the pulmonary artery pressure declines to adult levels within a week or two after birth. This fall in pressure coincides with a gradual decrease in the smooth muscle mass of the pulmonary arteries and with a decreased responsiveness of these arteries to hypoxia and acidosis (6).

In certain congenital heart defects associated with increased pulmonary blood flow (for example, large ventricular septal defects and transposition of the great arteries) there is often an elevated pulmonary artery pressure.
pressure and calculated pulmonary vascular resistance. This may represent persisting medial hypertrophy in fetal pulmonary arterioles or may be a secondary response to high pulmonary blood flow combined with exposure of the pulmonary vasculature to systemic blood pressures. In transposition, systemic hypoxia and pulmonary artery alkalosis might further encourage a progressive rise in pulmonary vascular resistance. Left atrial hypertension can also result in an elevated pulmonary artery pressure and increased pulmonary vascular resistance, as in mitral stenosis.

Pneumonectomy diverts the entire cardiac output through the remaining lung. A left pneumonectomy increases the right pulmo-
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nary blood flow by approximately 75%. We hoped to preserve the greater reactivity of the lung in the newborn by increasing its flow at an early age. Such a preparation could then be used to study the effects of hypoxia, acidemia, alkalalemia and left atrial hypertension on the remaining pulmonary circulation.

We used airway hypoxia to test pulmonary vascular reactivity in our experimental animals. Its effect has been extensively studied in both anesthetized and unanesthetized animals of several species, including cats, dogs, calves, and humans. These studies have recently been reviewed by De Burgh Daly and Hebb (7). There now seems to be little doubt that all species studied react to an inspired oxygen concentration of 15% or less by active pulmonary vasoconstriction but that there is considerable variation in the degree of response within and between species.

Sodium pentobarbital may diminish the pulmonary vascular response to hypoxia in dogs (8). However, in our experiments we used sodium pentobarbital in both puppies and minipigs and concluded that its possible effect on the pulmonary vascular responsiveness can be safely ignored in making comparisons.

Rudolph et al. studied the effects of pneumonectomy, complete ligation of the left pulmonary artery, and partial occlusion of the left pulmonary artery in adult mongrel dogs and in mongrel puppies aged 1 to 2 months. Serial observations were made on the changes in pulmonary artery pressure in these animals. Acute occlusion produced only insignificant changes in pulmonary artery pressure. One to two days after pneumonectomy or occlusion there was a rapid increase in pressure followed by a further gradual increase reaching somewhat higher levels in the puppies than in the adult dogs. Unexplained was the subsequent fall in pulmonary artery pressure in one adult dog and two puppies. Medial hypertrophy was found at histological examination of the lungs subjected to increased flow.

Massion and Schilling (9) studied 40 mongrel puppies that had pulmonary resection 1 to 7 days after birth. Twelve survivors were raised to maturity and then studied at 16 months of age. Their puppies also showed significant increases in pulmonary artery pressure over an assumed standard normal value of 18 mm Hg; these increases were of the same order as those found by Rudolph.

We were unable to confirm these results in puppies. Our animals showed no significant increase in pulmonary artery pressure either immediately or over a period of 16 weeks. Furthermore, the puppies with pneumonectomy proved no more sensitive to alveolar hypoxia or pulmonary artery acidemia than control puppies of the same age.

This difference might be explained by the difference in breed. The pulmonary vasculature of our beagles was hyporeactive compared with a hyperreactive vasculature in the mongrel puppies. Some of Rudolph's measurements were on conscious dogs, whereas ours were all anesthetized; however, his findings were also present under anesthesia. Unlike Massion and Schilling, we were unsuccessful in doing pneumonectomies in animals less than 1 month of age. Attempts to increase flow through the left lung by more than 100% by right pneumonectomy were also unsuccessful. No puppy survived this procedure, all dying within a few hours with an acutely hemorrhagic and edematous left lung, presumably due to overperfusion.

We therefore turned to Pitman Moore minipigs. Although knowledge of circulatory physiology in swine is somewhat fragmentary, they do show a much wider range of pulmonary vascular reactivity than dogs. Available studies suggest that the pulmonary artery pressure in pigs is relatively higher than in man, dog, or cat, but relatively lower than that characteristic of ruminents. Attinger and Cahill (10) have explained this on the basis of the relatively small pulmonary arterial tree and low distensibility of the pulmonary artery bed in pigs.

However, Evans et al. (11) found a systolic pulmonary artery pressure of 20 to 30 mm Hg in 14-day-old piglets. The pulmonary circulation in pigs appears to react briskly to narcotics.
and pulmonary artery pressure rises with age and stress. At high altitude, pigs develop right ventricular hypertrophy, which suggests a pulmonary vasoconstrictor response to hypoxia (12).

We found a mean pulmonary artery pressure of 19 ± 2.7 mm Hg in normal minipigs about 3 months old under light general anesthesia. This is somewhat higher than that observed in puppies. Pneumonectomized minipigs of a comparable age developed a significantly higher resting mean pulmonary artery pressure at similar blood flows, probably related to the low distensibility of the pigs’ pulmonary vascular bed. Disappointingly, the pneumonectomized animals did not prove more sensitive to hypoxia than were their normal age peers. They did show a progressive increase in pulmonary artery pressure with time. Histological studies of the lungs in the pneumonectomized animals showed grade I (medial hypertrophy) changes only (13).

Our results suggest that progressive pulmonary hypertension can be produced by left pneumonectomy in minipigs but not in beagle puppies. The vasculature of the remaining lung in both minipigs and beagle puppies is no more responsive to acute alveolar hypoxia than the pulmonary vasculature of normal animals. The minipig, however, is more reactive to hypoxia than is the beagle puppy and is a better animal to use for studies of pulmonary vascular response.

Acknowledgment
Gratitude is expressed to Drs. W. Zingg and John Burrington, who performed the pneumonectomies.

References
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Circ Res. 1971;28:397-402
doi: 10.1161/01.RES.28.4.397

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