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The Effect of Aminophylline on Diaphragm Blood Flow in the Piglet

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ABSTRACT. The effect of aminophylline on diaphragmatic blood flow was investigated in two groups of newborn piglets. Six animals were studied during spontaneous breathing and seven additional animals were paralyzed and ventilated to assess the effect of aminophylline on blood flow to the nonworking diaphragm. Arterial blood gases and pH, cardiac output, and diaphragmatic blood flow were measured before and 20 min after infusion of 20 mg/kg aminophylline. Blood theophylline concentrations averaged 117 μmol/L (21 μg/mL) in both groups of animals. Heart rate increased significantly in all animals. Cardiac output increased significantly only in spontaneously breathing animals. Aminophylline had no effect on blood flow to the costal or crural portions of the diaphragm in either the paralyzed or spontaneously breathing animals. (*Pediatr Res* 26:196–199, 1989)

Abbreviation

$\dot{Q}di$, diaphragmatic blood flow

Methylxanthines, and in particular the theophylline preparations, have been proposed as adjunctive therapy in weaning premature infants from mechanical ventilation (1, 2). In addition to their bronchodilatory effect (3–5) and central respiratory stimulation (6–9), methylxanthines improve diaphragmatic force output (10–13) and ameliorate the effects of diaphragmatic fatigue (10, 14–16) in adult humans and experimental animals. Similar information is not available for the newborn animal or infant.

Previous studies have suggested that aminophylline improves diaphragmatic function via a direct effect on muscle fiber contractility (17–19). It may also improve diaphragmatic contractility and protect from fatigue by increasing muscle blood flow (20, 21) and, thereby, substrate delivery (22). This study was designed to determine whether aminophylline has a primary effect on blood flow to the diaphragm in the piglet.

MATERIALS AND METHODS

A total of 13 farm-bred Yorkshire piglets were studied between 10 and 40 d of age; they weighed 2.7 to 5.2 kg. Only healthy animals with a respiratory rate of 15–30 breaths/min, a Pa_{O_2} of more than 8.0 kPa (60 torr) in room air, and a Pa_{CO_2} equal to or less than 6.7 kPa (50 torr) were accepted for study. The animals were anesthetized with sodium pentobarbital (15 mg/kg intravenously) and studied in the supine position. Subsequent doses of anesthesia were given as needed to prevent jaw clonus.

The trachea was surgically exposed and a metal tracheostomy tube was inserted. A femoral artery and vein were cannulated to monitor blood pressure, assess arterial blood gases and pH, and administer drugs. Arterial pH and blood gas tensions were measured with a Corning 168 blood gas analyzer (Corning Glass Works, Medfield, MA). Rectal temperature was continuously monitored (Telethermometer 43TA, Yellow Springs Instrument, Yellow Springs, OH) and maintained between 38.5 and 39.5° C. (23) with a radiant warmer. A 5 French thermodilution catheter was placed into the left branch of the pulmonary artery via the right external jugular vein under fluoroscopy. Cardiac output determinations were made in triplicate using a 9520A Cardiac Output computer (American Edwards Laboratories, Irvine, CA). Radiolabeled microspheres were used to measure diaphragmatic blood flow utilizing the reference sample method described by Heymann *et al.* (24). A polyethylene catheter (PE 90) was advanced into the left ventricle via the left carotid artery under fluoroscopic guidance for microsphere injection. Location of catheter tip was confirmed by pressure tracing and at postmortem. A second polyethylene catheter was placed into a femoral artery for withdrawal of the reference sample using a calibrated withdrawal pump (Harvard Apparatus, Millis, MA). The 15-μ microspheres labeled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, or ^{141}Ce (DuPont, Wilmington, DE) were used in these experiments. Approximately 1 million microspheres were diluted to a total volume of 2 mL with heparinized 0.9% saline. Before injection, the microsphere solutions were sonicated for 2 min, then shaken with a vortex mixer for 2 min. The microspheres were injected via the left ventricular catheter over a 30-s period whereas a simultaneous arterial reference sample was withdrawn from the aorta at a rate of 4.12 mL/min for 2 min, beginning 10 s before initiation of the injection. At the end of the experiment, the animal was killed with an overdose of sodium pentobarbital. The diaphragm, both kidneys and the lungs were removed and all fat was excised. The central tendon was removed from the diaphragm and the costal and crural components were separated and weighed. Tissue specimens were counted with a gamma scintillation spectrometer (Minaxi 5000 series, Packard, Laguna Hills, CA). Comparative blood flow to right and left kidneys was used as an index of adequate microsphere mixing within the left ventricle. Only those injections that resulted in a less than 15% difference between the two kidneys were used in analysis (24). Diaphragmatic vascular conductance was calculated by dividing the tissue blood flow values by the mean blood pressure measurement and was then expressed as $mL \cdot (g \cdot min \cdot mm\ Hg)^{-1} \times 10^3$.

Aminophylline was diluted in 5% dextrose in water before

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being administered intravenously in a dose of 20 mg/kg over a 10-min period. Postaminophylline studies were performed 20 min after the completion of the infusion and a blood sample for theophylline concentration was obtained at that time.

Two groups of animals were studied: in one group ($n = 6$), animals were allowed to breath 50% O_2 spontaneously. A hot wire anemometer was used to measure tidal flow and the flow signal was integrated (Hewlett Packard 8815 A, Palo Alto, CA) to determine tidal volume. In a second group ($n = 7$), animals were paralyzed with pancuronium bromide (0.3 mg/kg) and mechanically ventilated with an F_{IO_2} of 0.5 and a fixed volume ventilator with a tidal volume of 12–15 mL/kg. Frequency was adjusted to maintain P_{aCO_2} between 4.0–6.7 kPa (30–50 torr) (Harvard Apparatus, Millis, MA). Additional doses of pancuronium were given as needed to prevent spontaneous breathing. This group served as a control for variations in $\dot{Q}di$ that might occur with fluctuations in spontaneous ventilation.

Results are presented as mean ± SD unless otherwise noted. Differences in measured variables within a group were determined by the Student's t test (Minitab Release 80.1, Pennsylvania State University, University Park, PA, 1980).

Experimental protocol. The protocol was identical for both animal study groups. After a 30-min stabilization period, baseline hemodynamic (heart rate, blood pressure, cardiac output) and ventilatory (respiratory frequency, tidal volume) measurements were made and microspheres were injected. A total of 20 mg/kg of aminophylline was administered and the above measurements were repeated at 20 min postinfusion. Blood was drawn for a theophylline concentration, the animal was killed, and tissues were prepared for microsphere analysis.

RESULTS

Serum theophylline concentrations averaged 112 ± 4.4 μmol/L (20.2 ± 0.8 μg/mL) in paralyzed and 119 ± 2.8 μmol/L (21.5 ± 0.5 μg/mL) in spontaneously breathing animals. At these levels, aminophylline had no effect on arterial blood gas values in either group (Table 1).

Heart rate increased in both groups after aminophylline administration whereas mean BP was unchanged (Table 2). An increase in cardiac output after aminophylline reached statistical significance in spontaneously breathing animals but not in paralyzed animals. Aminophylline increased minute ventilation 40% ($p = 0.041$) in the spontaneously breathing animals.

Aminophylline had no effect on costal or crural diaphragmatic blood flow (Fig. 1). Vascular conductance of the total diaphragm, although higher in the spontaneously breathing versus paralyzed animals at baseline ($3.4 \pm 0.5 \times 10^3$ mL·(g·min·mm Hg)$^{-1}$ *versus* $1.3 \pm 0.2 \times 10^3$ mL·(g·min·mm Hg)$^{-1}$), was not altered by aminophylline administration.

DISCUSSION

Aminophylline has no effect on diaphragmatic blood flow or vascular conductance in the nonfatigued piglet diaphragm whether working (spontaneously breathing) or resting (paralyzed). Aminophylline increased minute ventilation in the spontaneously breathing animals as expected (7). Both groups of animals manifested the expected cardiovascular effects of the drug (25).

Skeletal muscle fatigue, and presumably diaphragmatic fatigue, occurs when the metabolic demands of the muscle exceed the nutrient supply (20, 26, 27), with blood flow being one major determinant of that supply. Thus, the ability to increase diaphragmatic blood flow might be expected to prevent the development of or treat already established diaphragmatic fatigue (22). Several nonspecific vasoactive drugs do improve diaphragmatic blood flow. Bundy *et al.* (28) found that amrinone may improve endurance as a direct effect of its diaphragmatic blood flow augmentation. Similarly, Scharf *et al.* (29) found that isoproterenol resulted in an increase in diaphragmatic blood flow at a given level of diaphragmatic activity when blood pressure was controlled.

Methylxanthines may also have direct vasodilating properties and, in part, improve muscle contractility by augmenting blood flow (30, 31). Oglivie *et al.* (30) demonstrated that in human adult volunteers, aminophylline at a blood concentration of 28–111 μmol/L (5–20 μg/mL) resulted in a dose-dependent increase in forearm blood flow and a decrease in forearm vascular resistance in subjects at rest. In adult dogs, Komarek *et al.* (31) found that theophylline resulted in a significant decrease in total peripheral vascular resistance, accompanied by a 10% increase in cardiac output. Similarly, Kariya *et al.* (32) found that aminophylline resulted in a decrease in perfusion pressure without changing $\dot{Q}di$ and concluded that aminophylline decreases diaphragmatic vascular resistance.

The values obtained in this study for diaphragmatic blood flow in the quietly breathing neonatal piglet are similar to pre-

Table 1. *Arterial blood gas and pH determinations (mean ± SD)*

		P_{aCO_2}		P_{aO_2}	
	pH	kPa	(torr)	kPa	(torr)
Paralyzed ($n = 7$)					
Before aminophylline	7.40 ± 0.09	4.5 ± 0.9	(34 ± 7)	30.3 ± 3.9	(227 ± 29)
After aminophylline	7.41 ± 0.08	4.9 ± 1.6	(37 ± 12)	30.4 ± 3.7	(228 ± 28)
Spontaneously breathing ($n = 6$)					
Before aminophylline	7.38 ± 0.04	5.7 ± 0.9	(43 ± 7)	22.8 ± 6.1	(171 ± 46)
After aminophylline	7.39 ± 0.04	5.5 ± 0.5	(41 ± 4)	24.7 ± 6.0	(185 ± 45)

Table 2. *Hemodynamic and ventilatory measurements (mean ± SEM)*

	Heart rate (bpm)	Mean BP (mm Hg)	Cardiac output (mL/kg/min)	Frequency (breaths/min)	Minute ventilation (mL/kg/min)
Paralyzed animals ($n = 7$)					
Before aminophylline	192 ± 15	99 ± 5	196 ± 18	28 ± 1	317.7 ± 38.6
After aminophylline	243 ± 12*	91 ± 5	214 ± 27	28 ± 1	321.3 ± 45.9
Spontaneously breathing animals ($n = 6$)					
Before aminophylline	186 ± 9	88 ± 11	263 ± 19	30 ± 4	284.4 ± 45.2
After aminophylline	256 ± 14*	87 ± 9	296 ± 18*	34 ± 5	398.4 ± 54.4*

* $p < 0.05$ compared to baseline value.

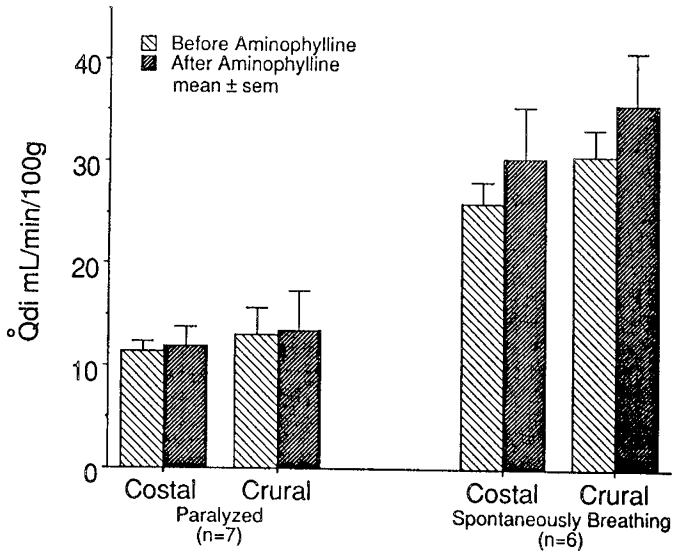

Fig. 1. Effect of aminophylline on diaphragmatic blood flow in paralyzed ventilated and in spontaneously breathing animals. No differences in blood flow was noted between the costal and crural segments of the diaphragm in either group of animals. No significant differences were noted after aminophylline administration.

viously published estimates of Q̇di that were based on a variety of techniques applied to a number of different species (28, 33–36). In contrast to the studies involving adult study subjects, our data point to the conclusion that aminophylline has no vasodilatory effect in the piglet diaphragm. Additionally, we were able to confirm a significantly higher baseline Q̇di in the animals who were spontaneously breathing. The latter data support the concept that one primary regulator of diaphragmatic blood flow is the metabolic demand of the muscle, the chief element of which is the level of contractile activity.

Several possibilities exist to explain the contrasting results of this study in the piglet as compared to those in adult experimental animals (31, 32). First, the piglet diaphragm, like that of other newborn mammalian species (37, 39, 40) is structurally and enzymatically different than its adult counterpart (37–40). The developing diaphragm has a much higher proportion of type IIA and a much lower proportion of type I and type IIB fibers; there are a significant number of type IIC fibers, which are completely absent in the adult diaphragm (37). In addition, the cross-sectional area of all fiber types is smaller in the neonatal as opposed to the adult diaphragm (37–40). This may have functional implications relating to control of diaphragmatic blood flow; it has been speculated that larger fibers may be more sensitive to limitations of substrate delivery due to greater diffusion distances (38).

The lack of increase in blood flow associated with the increase in ventilation induced by aminophylline in the spontaneously breathing animals is mostly likely a reflection of the relatively minor metabolic demand presented by the 40% increase in ventilation. Indeed, our findings confirm those of Rochester and Bettini (35) who found that during quiet breathing, diaphragmatic blood flow had no significant relationship to minute ventilation in adult dogs. This study demonstrates that in the young piglet, aminophylline does not have any direct vasoactive influence resulting in changes in blood flow in either paralyzed or spontaneously breathing animals.

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