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Effect of Aminophylline on Diaphragmatic Contractility in the Piglet

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ABSTRACT. Minute ventilation, arterial blood gases, arterial pH, cardiac output, and transdiaphragmatic force generation, both during spontaneous ventilation and in response to phrenic nerve stimulation during airway occlusion at end expiration, were measured in nine anesthetized, tracheostomized piglets before and 30 min after parenteral infusion of 20 mg/kg aminophylline. Serum theophylline levels averaged 109 ± 21 μmol/L (19.7 ± 3.7 μg/mL) at 30 min postinfusion. No significant changes were noted in pH, blood gases, blood pressure, or ventilatory measures after aminophylline. Aminophylline infusion also had no effect on transdiaphragmatic force generation at any frequency of phrenic nerve stimulation studied. It is concluded that aminophylline has no effect on diaphragmatic contractility in the quietly breathing, nonfatigued piglet. (*Pediatr Res* 28: 196–198, 1990)

Abbreviations

Pdi, transdiaphragmatic pressure

Aminophylline has been shown to increase the force output of the diaphragm in adult human study subjects and animal models (1–13). This effect has been demonstrated for the nonfatigued (1–13) as well as the fatigued diaphragm (1, 6, 10–12). The results of these studies have been extrapolated to the treatment of adult patients with respiratory failure (6). More recently, it has been suggested that methylxanthines may have a role in improving diaphragmatic force output in newborn infants (14, 15). However, data relevant to the effectiveness of methylxanthines in promoting improved force output in the newborn are sparse. Moreover, a clear understanding of the mechanism of action is lacking (16–18). We hypothesized that aminophylline would improve diaphragmatic contractility as evidenced by an increase in the force-frequency curve. This study was designed to assess the direct effect of aminophylline on contractility of the nonfatigued piglet diaphragm.

MATERIALS AND METHODS

Nine piglets were studied. Their mean postnatal age was 25 ± 6 d and mean body wt, 4.58 ± 1.72 kg. Only healthy animals with a respiratory rate of 15–30 breaths/min, a partial pressure of arterial oxygen > 8 kPa (60 torr) in room air, and a partial pressure of the arterial carbon dioxide ≤ 6.7 kPa (50 torr) were accepted for study. The animals were anesthetized with an i.v. combination of chloralose (30 mg/kg) and urethane (150 mg/kg) and studied in the supine position. Subsequent infusions of anesthetic were used if the piglet developed jaw clonus. A tracheostomy was placed and connected to a two-way nonrebreathing valve (model 2384, Hans Rudolph, Inc., Kansas City, MO). Inspiratory flow was detected by a hot-wire anemometer and integrated to provide tidal volume. All animals breathed 50% oxygen-balance nitrogen throughout the study. A femoral artery catheter was placed to measure heart rate and blood pressure, and to obtain samples for blood gas and pH analysis. Rectal temperature was continuously monitored and maintained at 39 ± 0.5°C by a radiant warmer.

Force-frequency curves were generated by the transvenous phrenic nerve stimulation technique as previously described (19, 20). The phrenic nerves were stimulated via bilateral indwelling external jugular vein catheter electrodes with supramaximal voltage at 20, 30, 50, and 100 Hz. Transdiaphragmatic force generation (Pdi) during airway occlusion at end expiration was measured as gastric pressure (Pgas) minus proximal airway pressure (Paw): Pdi = Pgas − Paw (21–23). Proximal airway pressure was measured by a P10EZ (Spectramed, Inc., Oxnard, CA) pressure transducer connected to an 18-gauge needle inserted in the tracheostomy tube between the animal and the nonrebreathing valve. A thin-walled latex balloon containing 0.5 mL of air was connected to a polyethylene catheter (1.65-mm inner diameter) and placed in the stomach to measure gastric pressure with a similar transducer.

A preformed plaster cast covering the entire abdomen and lower third of the chest was applied during force-frequency curve generation to ensure a constant degree of diaphragmatic shortening. The piglets were allowed to breathe unrestricted between stimuli. Chest wall distortion, if present, was assessed by respiratory inductive plethysmography (Respitrace, Ambulatory Monitoring, Inc., Ardsley, NY). Distortion was quantified in arbitrary units. A Swan-Ganz thermal dilution catheter (5 French with 10-cm proximal port, American Edwards Laboratories, Irvine, CA) was placed in the pulmonary artery under fluoroscopic guidance to measure cardiac output. Measures of cardiac output were obtained in triplicate using 2 mL iced 5% dextrose in water. End-expiratory lung volume was measured in three animals during baseline and again at 30 min post aminophylline infusion using the nitrogen washout technique (24).

Experimental protocol. After a 30-min stabilization period, baseline data were collected including arterial blood gases and pH, ventilatory parameters (tidal volume, respiratory rate, minute ventilation), cardiac output, systemic blood pressure, heart rate, chest wall distortion, and end-expiratory lung volume (n = 3), and a force-frequency curve was generated. Aminophylline was then infused (20 mg/kg) over 10 min. Data including serum

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theophylline levels were again collected at 30 min after the theophylline infusion.

All values are given as the mean ± SD unless indicated. Statistical comparisons were made using analysis of variance and the paired *t* test.

RESULTS

Data from the baseline and the 30-min study period are given in Table 1. No differences were noted in any ventilatory or hemodynamic measure from baseline to 30 min. Spontaneously generated Pdi was not affected by aminophylline (5.5 ± 1.6 cmH_2O at baseline *versus* 5.4 ± 2.0 cmH_2O 30 min post aminophylline infusion). Chest wall distortion during force-frequency curve generation at 30 min post theophylline infusion was not different from baseline at any frequency of phrenic nerve stimulation (Table 2). The results of force-frequency curve generation are shown in Fig. 1. No change was noted between baseline and 30 min post aminophylline infusion. End-expiratory lung volume did not change from baseline to 30 min postinfusion (18 ± 2 *versus* 17 ± 1 mL/kg, $n = 3$). Serum theophylline levels averaged 109 ± 21 μmol/L (19.7 ± 3.7 μg/mL).

DISCUSSION

The data generated by our study demonstrate that aminophylline has no effect on diaphragmatic contractility in the spontaneously breathing, nonfatigued, anesthetized piglet. Thus, the hypothesis of the study was rejected. To accept these results, the accuracy of the techniques used to determine force-generating capability of the diaphragm must first be assessed.

Critique. Our study was designed to focus on the diaphragm. Therefore, it was necessary to control central respiratory drive to the diaphragm and exclude the possibility of altered CNS output (25, 26). Accordingly, bilateral phrenic nerve stimulation was used to assess diaphragmatic function as previously described (19, 20, 27). The transdiaphragmatic pressure generated during phrenic nerve stimulation at end-expiratory lung volume was used as a measure of force-generating capacity.

Previous work in our laboratory has demonstrated that proximal airway pressure is superior to esophageal pressure as an estimate of intrapleural pressure during phrenic nerve stimulation (21–23). Accordingly, the measurement of transdiaphragmatic pressure in this model used proximal airway rather than esophageal pressure as an estimate of intrapleural pressure.

The force generated by the diaphragm for a given level of phrenic nerve input is a function of its length and geometry (28). Both of these factors are dependent on chest wall configuration and lung volume. Changes in chest wall configuration were minimized by the application of a snug-fitting plaster cast to the entire abdomen and lower third of the rib cage during Pdi measurement at end-expiratory lung volume. This was confirmed by lack of change in chest wall distortion as assessed by respiratory inductive plethysmography. Additionally, data generated as a subset of study animals suggest that end-expiratory lung volume did not change after aminophylline. Therefore, the length and geometry of the diaphragm were assumed not to be altered by aminophylline.

Methylxanthine effect on diaphragm. The methylxanthines are thought to affect muscle function by altering calcium ion flux across the cell membrane, including the sarcoplasmic reticulum and the T-tubular system (2, 16, 29, 30). Theophylline also causes hyperpolarization of the resting membrane potential, whereas caffeine has no effect (31). Both drugs raise intracellular pH (32), which affects calcium ion reuptake (33).

The reason for the lack of effect of aminophylline on diaphragmatic contractility in our study is not clear. The blood levels of theophylline achieved may not have been adequate to result in improved diaphragmatic contractility in the piglet. Because this study was designed to evaluate diaphragmatic function in a manner that could be extrapolated to the human neonate, the dosage of aminophylline used was calculated to result in serum levels at the high end of the acceptable clinical range (34). Indeed, Singrist *et al.* (8) demonstrated that aminophylline increased diaphragmatic contractility in a dose-related manner in the anesthetized adult dog. However, the peak effect was found at 80 mg/kg, a dosage 4 times greater than clinically therapeutic in adult humans. An alternative explanation may relate to subcellular structural differences. For example, the sarcoplasmic reticulum and the T-tubular system are incompletely developed in the newborn (35). Thus, cellular components thought to be key in the excitation-contraction phase may be unable to respond to the proposed drug effect on calcium flux phenomena.

Comparison to studies in adults. Our data are in conflict with investigations that demonstrated beneficial effects of methylxanthine drugs on diaphragmatic contractility in adult humans and animals. Aubier *et al.* (1) and Supinski *et al.* (9) both demonstrated a beneficial effect of methylxanthine drugs on diaphragmatic force output in adult humans. Sigrist *et al.* (8) demonstrated that aminophylline increased diaphragmatic contractility in a dose-dependent fashion in nonfatigued adult dogs. Similar findings have been demonstrated during metabolic acidosis (5) and hypercapnia (4) and in fresh and fatigued diaphragm in adult hamsters (12), adult rat diaphragms (36), adult human patients with chronic obstructive pulmonary disease (6), adult guinea pigs (13), and adult dogs (3). The beneficial effect of methylxanthine drugs on diaphragmatic contractility was apparent despite the use of anesthesia in some of the studies (2–5, 8). All these studies concluded that methylxanthine drugs would be of benefit in patients with diaphragmatic failure or fatigue.

Other investigators have not found improvement in diaphragmatic muscle force generation after methylxanthine drug administration. Paton *et al.* (37) found no improvement in ventilatory muscle strength or endurance in normal adult humans. In normal volunteers and in patients with chronic obstructive pulmonary disease, Belman *et al.* (38) could only demonstrate small improvements in ventilatory endurance thought to be of little significance. Foxworth *et al.* (17) and Kongragunta *et al.* (39) also found no improvement in diaphragmatic force generation in patients with severe chronic obstructive pulmonary disease. These studies all questioned the efficacy of methylxanthine drugs used to improve diaphragmatic function.

More recent investigations suggest that methylxanthines may result in adverse effects on diaphragmatic function. Miller and Reid (40) found that theophylline did not delay the onset of fatigue in the rat diaphragm and, indeed, inhibited force recovery after fatigue. Haack *et al.* (41) demonstrated that theophylline accelerated fatigue of isolated rat diaphragm muscle strips. Theophylline enhanced fatigue in hamster diaphragm (42, 43) in addition to slowing relaxation in the fatigued state (16). Thus,

Table 1. *Effect of aminophylline on cardiopulmonary measurements**

	HR	CO	BP	Vt	$\dot{V}e$	Pa_{O_2}	Pa_{CO_2}	Theophylline level
Preamino	175 ± 38	216 ± 40	96 ± 6	9.0 ± 1.9	182 ± 37	191 ± 29	43 ± 4	
Postamino	196 ± 67	211 ± 36	101 ± 9	10.2 ± 2.2	207 ± 85	175 ± 41	47 ± 6	109 ± 21 (19.7 ± 3.7)

* Preamino, baseline values before aminophylline infusion; Postamino, values 30 min postaminophylline infusion; HR, heart rate in beats/min; CO, cardiac output in mL/kg/min; BP, mean blood pressure in mm Hg; Vt, tidal volume in mL/kg; $\dot{V}e$, minute ventilation in mL/kg/min; Pa_{O_2}, partial pressure of arterial oxygen in torr; Pa_{CO_2}, partial pressure of arterial carbon dioxide in torr. Serum theophylline levels in μmol/L (μg/mL). All values are mean ± SD. No significant differences were noted between baseline and postinfusion measures.

Table 2. *Chest wall distortion**

	20 Hz	30 Hz	50 Hz	100 Hz
Preamino	4 ± 2	4 ± 3	5 ± 3	5 ± 3
Postamino	4 ± 2	5 ± 3	5 ± 4	6 ± 5

* Chest wall distortion in arbitrary units as obtained by inductive plethysmography. Hz, frequency of phrenic nerve stimulation during force frequency curve generation. Mean ± SD.

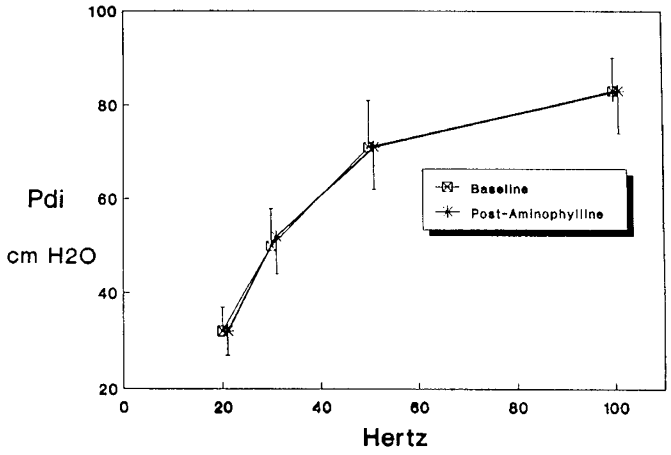

Fig. 1. No change in the force-frequency curve was noted from baseline (*thin line*) to 30 min post aminophylline infusion (*thick line*). Values are mean ± SEM. Values at 30 min post aminophylline are slightly offset for clarity. Hertz, frequency of phrenic nerve stimulation. Pdi, given in cm of water.

there are data suggesting that methylxanthines may adversely affect diaphragmatic force output. Whether methylxanthine drugs would result in improved diaphragmatic contractility in the absence of anesthesia cannot be answered by our study. Based on the above information, it would seem that a clear idea as to the efficacy of methylxanthines to augment diaphragmatic contractility during weaning from mechanical ventilatory support is lacking, and most certainly there are few data to support their use in the neonate.

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