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Aminophylline Reduces Hypoxic Ventilatory Depression: Possible Role of Adenosine

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ABSTRACT. Newborn infants and animals typically exhibit a paradoxical ventilatory response to hypoxia. The depressive phase of the response has not been adequately explained. It has been suggested that hypoxia may cause the release of inhibitory neuromodulators which depress ventilation. We have postulated that the nucleoside, adenosine, may be involved because 1) it is rapidly released during hypoxia, 2) it depresses ventilation, and 3) theophylline, a competitive inhibitor, has successfully been used to treat apnea of prematurity. Herein we describe the effects of aminophylline on ventilation during hypoxia in the spontaneously breathing newborn piglet administered both rapidly after ventilatory depression has occurred (bolus) and before the onset of hypoxia (pretreatment). Ten percent oxygen breathing produced a typical biphasic ventilatory response. The decrease in minute ventilation was caused by a decrease in both tidal volume and respiratory frequency. The bolus administration of aminophylline reversed the depression in minute ventilation ($p < 0.001$) by increasing tidal volume ($p < 0.002$). Pretreatment with aminophylline decreased the amount of ventilatory depression ($p < 0.05$) by preventing a decrease in respiratory frequency. We conclude that aminophylline, an adenosine antagonist, reduces the decrease in ventilation which occurs during hypoxia in the newborn. We speculate that adenosine may play a role in hypoxic ventilatory depression and respiratory control in the newborn. (*Pediatr Res* 19: 706-710, 1985)

Abbreviations

V_e , minute ventilation
 f , respiratory frequency

V_t , tidal volume
 HR, heart rate
 BPs, systolic blood pressure

The paradoxical ventilatory response to hypoxia has been well documented in the newborn animal and human, and may contribute to the etiology of neonatal apnea. The neonate typically exhibits a biphasic ventilatory response to hypoxia (1-3) characterized by a brief increase in V_e , followed by a sustained decrease which is further accentuated by the resumption of normoxic or hyperoxic gas inhalation. The initial increase in ventilation has conventionally been explained as reflecting peripheral chemoreceptor stimulation. The subsequent decrease, however, has not been satisfactorily explained. A failure of peripheral chemoreceptors is probably not involved (4-6) but a number of other mechanisms have been suggested including alterations in pulmonary mechanics (7), and hypoxic depression of central respiratory centers (8).

Central depression of respiration might be caused by a decrease in metabolic rate (9), alterations in cerebral blood flow (10), rapid exhaustion of facilitatory neurotransmitter stores (11-13), or the release of rapid acting inhibitory neuromodulator such as endogenous opioids (14) or GABA (15). We have recently postulated that the nucleoside, adenosine, may also be a candidate as an inhibitory neuromodulator (16, 17). Our postulate is based on three lines of evidence: 1) theophylline, a specific competitive inhibitor of adenosine, is effective in treating neonatal apnea (18); 2) adenosine is rapidly released in the brain during hypoxia (19); and 3) adenosine depresses ventilation when administered systemically or applied to the brainstem (20). Herein we describe the effects of theophylline on the hypoxic ventilatory response both when administered prior to the onset of hypoxia (pretreatment) and when given rapidly during hypoxia (bolus).

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MATERIALS AND METHODS

The hypoxic newborn piglet was chosen as an experimental model because recent investigations have determined that the piglet exhibits a biphasic hypoxic ventilatory response similar to the human infant (10). A total of 25 newborn piglets less than three days of age and weighing 1082.2 ± 302.4 g (mean $\pm$ SD) were obtained from local farms on the day of study. All animals were lightly anesthetized with intramuscular ketamine (15 mg/kg) and xylazine (1.5 mg/kg) and intubated with a 2.5 to 3.5 mm cuffed endotracheal tube. The endotracheal tube was attached to a breathing circuit which provided a continuous supply of heated, humidified gas. A system using solenoid valves was designed to allow a rapid change from room air to a predetermined gas mixture without interrupting or changing flow. Expiratory flow was measured with a pneumotachygraph (Fleisch no. 0000) placed between the endotracheal tube and the breathing circuit and connected to a differential pressure transducer (Validyne MP-45-18-87) and was electronically integrated to volume.

A catheter as placed in the femoral artery, infused with normal saline at 3 ml/h, and connected to a transducer (Statham P23DB) for continuous blood pressure measurements. The ECG and HR were monitored using standard extremity electrodes and skin temperature was automatically adjusted under a servo-controlled radiant heat source to maintain core temperature between 38 and 39° C., the normal body temperature for neonatal piglets. Arterial blood gases were analyzed using a clinical blood gas system (IL-113). Measurements of f , inspiratory and expiratory flow, expired volume, ECG, heart rate, and blood pressure were continuously displayed on a strip chart recorder (Beckman Dynagraph). From these data, mean V_e , f , V_t , HR, and BPs were calculated for each minute of study.

During each experiment, anesthesia was maintained by intramuscular injections of ketamine (7.5 mg/kg) and xylazine (0.75 mg/kg) given every 20 to 30 minutes. In a separate group of five piglets maintained in room air for 3 h, this method did not affect V_e , f , V_t , or respiratory pattern.

The effect of pretreatment with theophylline on the hypoxic ventilatory response was studied in 11 piglets. Each animal was used as its own control and a control hypoxic trial was followed by a treatment trial. The control trial consisted of rapidly decreasing the inspired oxygen concentration to 0.10 and maintaining this concentration for 6 min. Normoxia was then resumed and maintained for another 5 min. Theophylline in the form of aminophylline (15 mg/kg) was then administered intra-arterially over 10 min, and after 30 min, the hypoxic trial was repeated. We determined in a separate group of five piglets, that aminophylline, at a dose of 15 mg/kg, administered over 10 min, resulted in theophylline levels of 12 to 15 μ g/ml 30 min after the completion of the injection. Arterial blood gases were obtained from the femoral artery before and during the 6th min of hypoxia. The percentage change in V_e , f , and V_t at 2 and 6 min and the absolute changes in arterial blood gas values, HR, and BPs at 6 min after the onset of hypoxia were compared for the control and pretreatment trials using paired t tests corrected for the effect of multiple tests according to the method of Bonferroni (21).

The effects of a bolus injection of aminophylline administered during hypoxia was evaluated in 14 additional animals as part of another study. In all 14 animals, hypoxia was first induced by rapidly decreasing the inspired oxygen concentration to 0.10. After 6 min of hypoxia, 7 animals received a bolus of 1.6 ml of aminophylline mixed in normal saline (15 mg/kg) and seven animals received 1.6 ml of a normal saline placebo, intra-arterially over 1 min. The animals were then observed for an additional 5 min before resuming normoxia. In another group of four animals, a bolus of aminophylline at a dose of 15 mg/kg, administered over 1 min resulted in theophylline levels of 17 to 28 μ g/ml. Arterial blood gases were obtained before and during the 6th min of hypoxia, and then 5 min after either the saline or the aminophylline injection. The 6th min of hypoxia was used as a

baseline and the percent changes in V_e , f , V_t , and the absolute changes in arterial blood gases, HR, and BPs were compared for the two groups 5 min after the bolus injection of either aminophylline or saline. Unpaired t tests were used to compare the means of each parameter.

The effect of theophylline on periodic breathing and respiratory irregularity was evaluated in both the pretreatment and bolus experiments. Periodicity was defined as alternating periods of breathing and apnea occurring at least twice sequentially during any 1-min period [adapted from Rigatto and Brady (2)]. Irregularity was defined as significant variation in both amplitude and frequency. In the pretreatment experiments, the 6th min of hypoxia of both the control and treatment trials was scored as either periodic or nonperiodic and either regular or irregular. In the bolus experiments, the 5th min after the aminophylline or saline bolus was similarly scored. The entire minute was scored for each variable according to the dominant pattern. The effect of hypoxia and aminophylline on the relative amount of periodic and irregular breathing was statistically evaluated using Fischer exact tests.

RESULTS

During hypoxia, breathing became irregular and some animals exhibited pronounced periodicity. Table 1 illustrates the effect of aminophylline on respiratory pattern. Both bolus ($p < 0.05$) and pretreatment ($p < 0.005$) with aminophylline appeared to prevent hypoxic respiratory irregularity with little effect on periodicity.

In the pretreatment experiments, hypoxia produced a typical biphasic response in the control trials, with a transient increase and then a sustained decrease in V_e , accompanied by corresponding changes in both f and V_t . Following the control hypoxic trial, the administration of 15 mg/kg of aminophylline was associated with a transient increase in V_e accompanied by an increase in both f and V_t . After 30 min, V_t was significantly greater than baseline (7.4 ± 1.5 versus 6.2 ± 1.3 ml/kg; $p < 0.05$). After pretreatment with aminophylline, hypoxia also caused an initial increase in V_e with accompanying increases in both f and V_t . These changes were not different from those observed in the control trials. The decrease in V_e , however, was reduced after treatment with aminophylline. The percentage changes in V_e , f , and V_t during the control and aminophylline pretreatment hypoxic trials are compared in Figure 1.

The difference between the decreases in V_e in the control and the aminophylline pretreatment trials was due to a difference in the changes in f . While there were no differences in the decreases in V_t , f decreased in the control hypoxia trials and remained elevated in the aminophylline trials. By the 6th min of hypoxia, f was $6.5 \pm 8.3\%$ below baseline in the control group compared to $11.0 \pm 10.8\%$ above baseline in the aminophylline group ($p < 0.05$).

The values for arterial blood gases, HR, and BPs before and after treatment with aminophylline during normoxia and the changes during the control and pretreatment hypoxic trials are shown in Table 2. Treatment with aminophylline during normoxia produced a significant elevation in PAO_2 and HR, but had little effect on $PACO_2$, pH, or BPs. In the control trials, 6 min of hypoxia was associated with a decrease in blood pressure and $PACO_2$ and an increase in pH and HR. After pretreatment with

Table 1. Hypoxic breathing pattern

% of epochs	Control	Aminophylline	<i>p</i>
Bolus			
Periodic	16.7	20.1	NS
Irregular	85.7	25.0	<0.005
Pretreatment			
Periodic	24.1	18.6	NS
Irregular	86.2	48.1	<0.005

Fig. 1. Pretreatment experiments. Percentage changes in VE , f , and V_t during hypoxia before (open circles) and after (closed circles) treatment with aminophylline. Changes from baseline (prehypoxia) are compared. Values are means $\pm$ SEM.

Table 2. Pretreatment experiments (mean $\pm$ SEM)

	Normoxia	Hypoxia	Difference
PAO₂			
Control	54.8 $\pm$ 2.3	24.5 $\pm$ 2.0	30.4 $\pm$ 3.3
Aminophylline	65.7 $\pm$ 4.3	25.2 $\pm$ 1.6	40.1 $\pm$ 5.9
<i>p</i>	<0.01	NS	<0.05
PACO₂			
Control	31.6 $\pm$ 1.8	26.5 $\pm$ 1.1	5.2 $\pm$ 1.3
Aminophylline	29.6 $\pm$ 1.9	23.6 $\pm$ 1.3	5.9 $\pm$ 1.2
<i>p</i>	NS	NS	NS
pH			
Control	7.44 $\pm$ 0.02	7.49 $\pm$ 0.02	0.01 $\pm$ 0.01
Aminophylline	7.48 $\pm$ 0.02	7.50 $\pm$ 0.04	0.01 $\pm$ 0.03
<i>p</i>	NS	NS	NS
HR			
Control	140 $\pm$ 8	163 $\pm$ 9	22.9 $\pm$ 5.6
Aminophylline	157 $\pm$ 8	191 $\pm$ 9	32.3 $\pm$ 4.5
<i>p</i>	<0.02	<0.001	NS
BPs			
Control	90 $\pm$ 3	73 $\pm$ 4	16.4 $\pm$ 3.3
Aminophylline	89 $\pm$ 3	78 $\pm$ 4	10.4 $\pm$ 2.2
<i>p</i>	NS	<.25	<0.05

aminophylline, hypoxia produced similar changes. When the changes in the control and pretreatment experiments were compared, PAO₂ decreased more and BPs decreased less after pretreatment with aminophylline.

The effects of the bolus administration of aminophylline and saline during hypoxia on Ve , f , and V_t are shown in Figure 2. Prior to the administration of aminophylline or saline, both groups exhibited typical biphasic responses and by 6 min of hypoxia ventilation was not different from prehypoxia baseline values. Bolus administration of aminophylline was associated with a significant increase in Ve and V_t when compared to the changes associated with the placebo. Although f increased after the administration of aminophylline, this change was not different from the increase associated with saline infusion.

The effect of aminophylline and saline, administered during hypoxia, on PAO₂, PACO₂, pH, BPs, and HR are illustrated in Table 3. When compared to the infusion of saline, aminophylline did not significantly improve PAO₂, but decreased PACO₂ and

Fig. 2. Bolus experiments. Percentage changes in VE , f , and V_t during hypoxia. After 6 min of hypoxia the animals were either treated with saline (open circles) or aminophylline (closed circles). Changes from 6 min of hypoxia are compared. Values are means $\pm$ SEM.

Table 3. Bolus experiment (mean $\pm$ SEM)

	Normoxia	Hypoxia ¹	Hypoxia ² + drug	Difference (2 - 1)
PAO₂				
Saline	58.0 $\pm$ 1.0	20.2 $\pm$ 0.7	19.0 $\pm$ 0.7	1.2 $\pm$ 0.4
Amino- phylline	56.6 $\pm$ 3.6	18.8 $\pm$ 1.3	19.8 $\pm$ 1.2	1.0 $\pm$ 1.1
<i>p</i>				NS
PACO₂				
Saline	38.8 $\pm$ 1.3	36.3 $\pm$ 1.4	38.9 $\pm$ 1.9	2.6 $\pm$ 0.9
Amino- phylline	36.3 $\pm$ 1.7	36.2 $\pm$ 2.1	34.8 $\pm$ 2.1	1.4 $\pm$ 0.8
<i>p</i>				<0.01
pH				
Saline	7.46 $\pm$ 0.01	7.49 $\pm$ 0.01	7.46 $\pm$ 0.02	0.03 $\pm$ 0.01
Amino- phylline	7.47 $\pm$ 0.02	7.47 $\pm$ 0.01	7.48 $\pm$ 0.01	0.01 $\pm$ 0.01
<i>p</i>				<0.02
HR				
Saline	121 $\pm$ 3	135 $\pm$ 3	141 $\pm$ 9	6.0 $\pm$ 2.9
Amino- phylline	121 $\pm$ 5	134 $\pm$ 8	159 $\pm$ 11	24.4 $\pm$ 5.9
<i>p</i>				<0.02
BPs				
Saline	79 $\pm$ 4	77 $\pm$ 4	77 $\pm$ 4	3.6 $\pm$ 2.6
Amino- phylline	83 $\pm$ 4	80 $\pm$ 5	81 $\pm$ 4	3.0 $\pm$ 4.7
<i>p</i>				NS

increased pH. The effect of aminophylline on BPs was not different than the effect of saline but HR increased more after aminophylline than after saline.

DISCUSSION

Our results confirm the reports of other investigators that the biphasic hypoxic ventilatory response is present in the spontaneously breathing newborn piglet (10). Our finding that during hypoxia, the increase and subsequent decrease in ventilation results from an increase followed by a decrease in both V_t and f , is also consistent with the work of other investigators (14). Our results demonstrate that the decrease in ventilation associated with hypoxia can be reversed by the rapid administration of aminophylline and partially prevented by pretreatment with aminophylline.

The bolus administration of aminophylline during hypoxia resulted in a significant increase in V_t and V_e . Treatment with aminophylline during normoxia also resulted in a significantly elevated V_t . This is consistent with other reports showing that during normoxia, xanthines cause a large increase in V_t with a smaller increase in f (22, 23). Although the respiratory stimulatory effects of methylxanthines have been well documented, the primary sites of action and neurochemical mechanisms remain uncertain. Investigators have suggested that xanthines could stimulate respiration by affecting respiratory muscle contractility (24), pulmonary mechanics (7), metabolic rate (25), the release of catecholamines (26), medullary blood flow (27), or by directly increasing central respiratory activity (22).

In a series of sophisticated studies in adult cats, Eldridge *et al.* (22), using phrenic nerve activity as an indicator of central respiratory activity, determined that the primary stimulatory action of aminophylline is probably not mediated via pulmonary mechanical factors, vagal reflexes, changes in arterial $PACO_2$, release of substances from the adrenal, or changes in whole body metabolic rate. Aubier *et al.* (28) have demonstrated that aminophylline improves diaphragmatic contractility in adult volunteers, and renders it less susceptible to fatigue. These authors have also suggested that the increase in V_e and V_t associated with aminophylline infusion is primarily caused by improved diaphragmatic efficiency (24). These data suggest that the primary stimulatory effect of methylxanthines on respiration involves both a direct stimulation of central respiratory centers and an improvement of diaphragmatic contractility.

Pretreatment with aminophylline had no effect on the initial increase in V_t and f caused by hypoxia, but prevented the subsequent decrease in f with little effect on V_t . A number of investigators have demonstrated that theophylline causes respiration to become more regular in infants with apnea of prematurity (23). In our model, pretreatment with theophylline regularized respiration during hypoxia. This suggests that the effect on respiratory frequency we observed may be secondary to the prevention of respiratory irregularity associated with hypoxia.

Our results disagree with those of Long and Lawson (29) who found that the pretreatment of neonatal piglets with aminophylline had no effect on phrenic nerve output during hypoxia. Although the reasons for the differences in our results are not clear, differences in the experimental models may be important. Although both studies were done with newborn piglets, our studies were carried out in fully intact, lightly anesthetized, spontaneously breathing animals, while the studies of Long and Lawson (29) were carried out in anesthetized, paralyzed, vagotomized animals, servo-ventilated to minimize changes in $PACO_2$. It is possible that differences in afferent input from central chemoreceptors and pulmonary receptors may have contributed to the differences in the results of the two studies. Another possibility is that our results reflect the effect of aminophylline on diaphragmatic fatigue during hypoxia. Aminophylline may have improved contractility resulting in a diminution in the decrease in V_e (28). Pretreatment with aminophylline, however,

prevented a decrease in f with little effect on V_t , suggesting a central, rather than a peripheral effect.

Our finding that pretreatment with aminophylline prevented the decrease in f rather than V_t during hypoxia suggests that methylxanthines may also interfere with mechanisms responsible for hypoxic ventilatory depression. It is possible that the primary respiratory effect of methylxanthines may involve one set of mechanisms, while the effect on hypoxic respiratory depression may involve another. Aminophylline could affect several of the suggested mechanisms of hypoxic ventilatory depression including changes in metabolic rate, exhaustion of facilitatory neurotransmitter stores, alterations in cerebral blood flow, and the release of adenosine.

Eldridge *et al.* (22) found that aminophylline increases phrenic nerve output in paralyzed adult cats without a concomitant increase in metabolic rate, while other investigators have reported increases in metabolic rate in spontaneously breathing human infants treated with aminophylline for apnea of prematurity (23). Eldridge *et al.* (22) suggested that the difference in these findings may be due to the neuromuscular blockade in their animals. In the unparalyzed animal, aminophylline may potentiate the contraction of skeletal muscle both by increasing tonic activity due to central neural mechanisms and by increasing muscle tension due to an effect on transmitters at the neuromuscular junction (30).

It is possible, therefore, that in spontaneously breathing animals, aminophylline may prevent a decrease in metabolic rate which may occur during hypoxia. Early reports of Cross *et al.* (9) demonstrated decreases in oxygen consumption during 15% oxygen breathing in human infants (1). Hill (31) demonstrated in newborn kittens, however, that the decrease in oxygen consumption does not occur during hypoxia when the animal is at thermoneutrality. In our studies, the skin temperature of all piglets was servo-controlled to maintain core temperatures between 38 and 39° C. It is unlikely, that a temperature-related decrease in metabolism is responsible for the decrease in ventilation we observed.

Xanthines may affect respiration by altering blood flow. Lawson and Long (10) have suggested that during hypoxia a combination of a vasodilatation, increased cardiac output, local increases in brain perfusion, and an increase in CO_2 carrying capacity may result in net CO_2 removal and thus a removal of hydrogen ions from brain chemosensory tissues. The resultant inhibitory central chemosensor input may then override peripheral chemosensor excitatory input resulting in a net decrease in ventilation. Xanthines are potent cerebral vasoconstrictors (32, 33), and aminophylline may affect ventilation by interfering with changes in cerebral perfusion that occur during hypoxia.

Xanthines may reduce hypoxic ventilatory depression by facilitating the release of catecholamines from the adrenal (26, 34). Infused catecholamines have been reported to stimulate ventilation (35). The levels of xanthines, however, required to release catecholamines are generally greater than those usually used clinically or in our experiments (32). Also, Eldridge *et al.* (22) were unable to blunt the stimulatory effect of xanthines by ablating the adrenals and Bhat *et al.* (36) were unable to detect any increase in urinary catecholamine metabolites in infants treated with aminophylline for apnea of prematurity. These reports suggest that it is unlikely that the effects we observed were due to an increase in circulating catecholamines.

We propose that the effects of aminophylline that we observed may be due to the competitive inhibition of the actions of the nucleoside, adenosine. In animal models, adenosine is rapidly released during hypoxia (19), and most is deaminated to inosine and hypoxanthine. This is consistent with reports that inosine, hypoxanthine, and xanthine—adenosine metabolites, can be measured in the cerebral spinal fluid of asphyxiated human infants (37). The properties of rapid release and uptake make adenosine an ideal candidate as a regulator of cerebral blood flow (4) and as a modulator of synaptic transmission (38). Also,

adenosine depresses the firing rate of cerebral neurons (39). It is possible, therefore, that during hypoxia, both central and vascular effects of adenosine could be involved in ventilatory depression.

In the newborn animal, the systemic administration of adenosine analogues causes physiologic changes similar to those found during severe, prolonged hypoxia, including bradycardia, hypotension, and apnea. Also, the direct application of adenosine analogues to the brainstem depresses ventilation (17, 20). An adenosine receptor inhibitor such as aminophylline would be expected to reverse these effects. In this study, pretreatment with aminophylline reduced the amount of hypoxic ventilatory depression and reduced the fall in BPs. Although HR did not fall during the brief exposure to hypoxia, the mean HR tended to be higher after pretreatment with aminophylline.

It has frequently been proposed that the stimulant effects of methylxanthines are secondary to an accumulation of cyclic AMP caused by an inhibition of nucleotide phosphodiesterase. Recent evidence, however, indicates that this may not be the major mechanism involved. Xanthines, even at maximal therapeutic levels, have been found to inhibit phosphodiesterase activity in many tissues by only 5 to 10% (40). Furthermore, methylxanthines in this concentration range have only rarely been observed to potentiate hormone-induced effects, even in those instances where other evidence exists for the involvement of cyclic AMP (32). Adenosine has also been shown to be a potent modulator of adenylate cyclase in many tissues. In the brain, adenosine appears to catalyze the production of cyclic AMP (13) while theophylline has been found to decrease levels of cyclic AMP (40). It is reasonable to postulate, therefore, that the effects of theophylline on V_E , HR, and BPs during hypoxia may be due to the inhibition of the actions of adenosine rather than the phosphodiesterase.

In conclusion, our results have shown that in the newborn piglet, the rapid infusion of aminophylline during hypoxia increases V_t and reverses ventilatory depression. Also, pretreatment with theophylline at low levels ($<15 \mu\text{g/ml}$) modifies the biphasic hypoxic ventilatory response, reducing the degree of ventilatory depression, primarily through a maintenance of respiratory frequency. We suggest that the effect of aminophylline on respiration during hypoxia may be the result of actions at more than one site and that the resultant respiratory pattern is a balance between excitatory and inhibitory inputs. Our results are consistent with the hypothesis that the effects of pretreatment with aminophylline on the hypoxic ventilatory response may be due to its inhibitory actions on adenosine receptors and speculate that adenosine may play a role in respiratory control.

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