Increase in ventilation caused by aminophylline in the absence of changes in ventral medullary extracellular fluid pH and carbon dioxide tension · · · ·

S JAVAHERI, J A M EVERS, L J TEPPEMA

From the Pulmonary Section and Department of Medicine, Veterans Administration Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA, and the Department of Physiology, Catholic University of Nijmegen, Nijmegen, The Netherlands

ABSTRACT   A study was designed to investigate the possibility that changes in ventral medullary extracellular fluid carbon dioxide tension (PCO_2) and hydrogen ion (H^+) concentration mediate the ventilatory stimulation induced by systemic administration of aminophylline. Six cats with peripheral chemodenervation (bilateral carotid sinus nerve and vagal neurotomy) were studied while anaesthetised with chloralose urethane and breathing spontaneously at a regulated, constant, and somewhat raised end tidal PCO_2. Variables were measured during steady state normoxaemia and 10 and 30 minutes after administration of 17 mg/kg aminophylline (mean (SD) blood concentration of theophylline 14·2 (1·5) mg/l). Aminophylline resulted in a significant and considerable increase in minute ventilation to 155% and 167% above baseline at 10 and 30 minutes. Mean (SD) values for arterial PCO_2 were 5·6 (0·6), 5·6 (0·6), and 5·4 (0·6) kPa at 0, 10, and 30 minutes. Values for the ventral medullary extracellular fluid PCO_2 were 7·6 (1·1), 7·5 (1·0), and 7·4 (1·0) kPa and for H^+ concentration 62·7 (9·3), 62·6 (9·3), and 63·5 (8·0) nmol/l at 0, 10, and 30 minutes. After aminophylline infusion the PCO_2 and H^+ concentration of the extracellular fluid did not differ significantly from baseline values. It is concluded that in spontaneously breathing, peripherally chemodenervated cats the considerable increase in ventilation that follows an infusion of aminophylline is not mediated by change in the ventral medullary extracellular fluid PCO_2 or hydrogen ion concentration.

Introduction

Methylxanthines are known to stimulate ventilation. They are used in the treatment of apnoea of preterm infants[1] and can restore the respiratory pattern to normal in patients with Cheyne-Stokes respiration.[2] Recent studies have shown that aminophylline increases hypoxic ventilatory drive.[3]

Methylxanthines may stimulate ventilation by one of several mechanisms.[3][4] A recent study[5] suggested that competitive inhibition of adenosine, which acts centrally as a respiratory depressant, might be responsible.

Address for reprint requests: Dr Shahrokh Javaheri, Pulmonary Section (111A), Veterans Administration Medical Center, 3200 Vine Street, Cincinnati, Ohio 45220, USA.

The specific aim of this study was to investigate the possibility that changes in ventral medullary extracellular fluid acid-base variables could mediate the stimulatory effect of aminophylline on ventilation. Recent studies[6][8] have suggested that brain carbon dioxide tension (PCO_2) may act independently from hydrogen ions to stimulate ventilation. Aminophylline has been thought to produce vasoconstriction of the cerebral vascular bed,[9][10] and if this is true the reduction in cerebral blood flow should increase cerebral PCO_2, assuming that the cerebral metabolic rate remains unchanged. We measured ventilation and the PCO_2 and pH of ventral medullary extracellular fluid before and after administration of aminophylline in anaesthetised, spontaneously breathing cats. The experiments were performed after carotid sinus nerves and vagi had been cut to remove any input from peripheral chemoreceptors.

Methods

ANIMALS AND INSTRUMENTS

We used six cats (of both sexes, weight 2–4·6 kg), which were being studied in a larger series of experiments.[11][12] They were premedicated with ketamine hydrochloride (25 mg intramuscularly) and anaesthetised with 1·5–2 ml chloralose urethane (50 mg chloralose and 250 urethane mg/ml) intravenously; two or three additional doses of 0·25 ml chloralose urethane were given during the operation procedures. In addition, subcutaneous atropine (0·25 mg) was given to decrease airways secretions and intravenous dexamethasone (5 mg in 5 ml saline) to lower the risk of brain oedema. The cats breathed spontaneously throughout the study. The trachea was cannulated and femoral arterial and venous catheters were inserted to monitor arterial blood pressure, sample arterial blood, and administer drugs intravenously. Rectal temperature was maintained constant by a heating table. Ventilation was measured spirometrically[16] through a closed respiratory circuit in which end tidal Pco_2 and oxygen tension (Po_2) could be independently maintained at the desired level. For measurement of arterial blood gases and pH, 1 ml arterial blood was obtained in 1 ml tuberculin syringes in which the dead space was filled with heparin. Arterial blood Po_2, Pco_2, and pH were determined immediately by appropriate electrodes at 37°C (pH/blood gas analyser, model 413, Instrumentation Laboratory, Lexington, Montana). The values were corrected to the animal's rectal temperature, and plasma bicarbonate ion (HCO_3^-) concentration was calculated with the Severinghaus calculator.

To expose the ventrolateral medullary surface, the upper trachea was removed and the dura mater opened and reflected sideways as described previously.[6] The pH of the extracellular fluid on the medullary surface was measured with combined glass electrodes with a flat surface (Ingold, Urdorf, Switzerland, LOT 403-M5, diameter 5 mm). The electrode was connected to a high impedance pH meter (Knick, model 645, Berlin), which had an isolated input. Calibration of the electrode was carried out in vitro with standard phosphate buffers at 37°C.

For measurement of the Pco_2 of the extracellular fluid on the medullary surface, a flat surface (diameter 3 mm) Pco_2 electrode (Microelectrodes Inc, Londonderry, New Hampshire, USA) was placed in close proximity to the pH electrode on the brain surface as described.[11][12] The electrode was connected to an amplifier with isolated input (Knick, model 645). The Pco_2 electrode was calibrated in vitro, in mock cerebrospinal fluid at 37°C tonometered with three gas mixtures containing about 4%, 6%, and 8·5% carbon dioxide. The concentration of carbon dioxide in the

three gas mixtures was determined by the Scholander technique. The temperature of the mock cerebrospinal fluid was kept at 37°C (the temperature probe used to measure medullary surface temperature being used). These electrodes were Nernstein.[12] In one electrode the slopes measured on five days varied from 57·5 to 59·5 (mean 58·8 (SD 0·74)) millivolts. The correlation coefficients of the linear regression equations relating in vitro Pco_2 values to the millivolt reading were invariably more than 0·99. Brain extracellular fluid HCO_3^- concentration was calculated from the Henderson-Hasselbalch equation on the basis of brain extracellular fluid Pco_2 and pH.[13]

Variables were recorded on a six channel strip chart recorder (Gould, Cleveland, Ohio) or a magnetic tape recorder (Bell and Howell 4020A), or both.

EXPERIMENTAL DESIGN

Carotid sinus nerves and cervical vagi were cut bilaterally in six spontaneously breathing cats. Steady state measurements of the following were made: arterial blood pressure, minute ventilation, tidal volume, end tidal Pco_2 and Po_2, Pco_2, pH, and temperature of the ventral medullary extracellular fluid, arterial blood gases and pH, and rectal temperature. Aminophylline 17 mg/kg (equivalent to 13·6 mg/kg theophylline) dissolved in 10 ml normal saline was infused intravenously over 10 minutes. The measurements were repeated 10 and 30 minutes after the beginning of the infusion of aminophylline.

We performed our experiments at a constant, mildly raised $Paco_2$ (average 5·6 kPa). In this way $Paco_2$ could be kept relatively constant and independent of changes in ventilation.

STATISTICAL ANALYSIS

Variables were analysed by analysis of variance for repeated measurements. The differences between mean values were assessed by multiple *t* tests and the significance level was adjusted by the Bonferroni method. Data are expressed as means with standard deviations in parentheses; p < 0·0175 was considered significant.

Results

The figure shows an original tracing of tidal volume, end tidal Pco_2 and Po_2, the Pco_2 and pH of the ventral medullary extracellular fluid, and arterial blood pressure in one cat before and after administration of aminophylline.

The table shows the mean values for arterial blood and extracellular fluid ventral medullary acid-base variables and the ventilatory measurements. There were no significant changes in Pao_2, $Paco_2$, or plasma [H^+] after aminophylline but the small fall in plasma
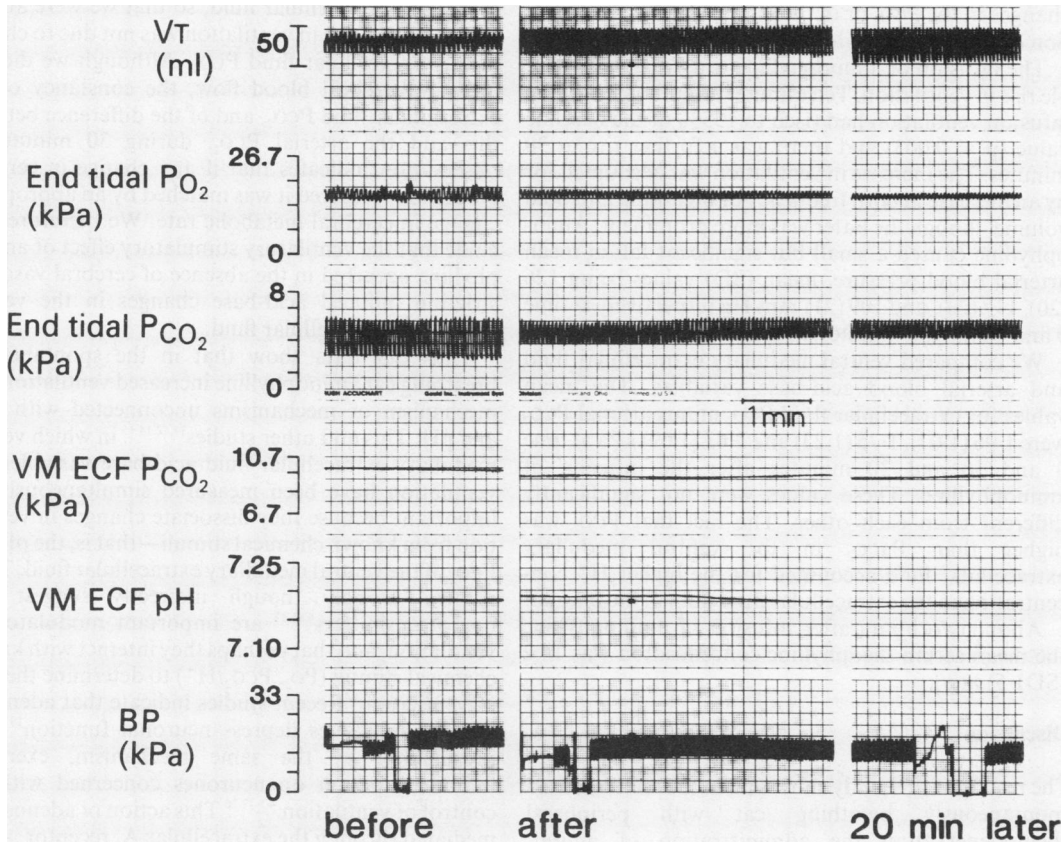
Tracings showing tidal volume (VT), end tidal oxygen and carbon dioxide tensions (Po$_2$ and Pco$_2$), ventral medullary extracellular fluid (VM ECF) Pco$_2$ and pH, and arterial blood pressure (BP) before and after administration of intravenous aminophylline. After aminophylline ventilation increased and more carbon dioxide had to be added to the inspiratory port to keep end tidal carbon dioxide constant. Initially, however, end tidal Pco$_2$ was somewhat lower than baseline, resulting in a rise in VM ECF pH and a fall in VM ECF Pco$_2$; with time, as end tidal Pco$_2$ increased, changes in VM ECF acid-base variables were minimised.

Mean (SD) values for ventral medullary extracellular fluid (VM ECF) acid-base variables and ventilatory measurements before and after aminophylline infusion in six anaesthetised spontaneously breathing cats

	Arterial blood					VM ECF				Ventilatory measurements		
Time (min)	Po$_2$ (kpa)	Pco$_2$ (kPa)	[HCO$_3^-$] (nmol/l)	[H+] (nmol/l)	pH	Pco$_2$ (kPa)	[HCO$_3^-$] (mmol/l)	[H+] (nmol/l)	pH	V̇ (ml/min)	F (/min)	VT (ml)
0	14·4 (0·9)	5·6 (0·6)	20·2 (2·2)	50·6 (8·0)	7·296	7·6 (1·1)	21·3 (3·0)	62·7 (9·3)	7·203	817 (251)	14·6 (3·2)	56·0 (13·7)
10	14·4 (1·2)	5·6 (0·6)	19·9 (1·7)	50·7 (9·1)	7·95	7·5 (1·0)	20·9 (2·5)	62·6 (9·3)	7·203	1268* (317)	17·7* (3·8)	72·2* (16·3)
30	14·5 (1·1)	5·4 (0·6)	19·2* (1·9)	50·8 (7·6)	7·294	7·4 (1·0)	20·4 (2·4)	63·5 (8·0)	7·197	1361* (325)	17·3* (2·9)	78·2* (14·8)

Ventilation (V̇) rose significantly 10 and 30 minutes after infusion of aminophylline. There were no significant changes in ventral medullary extracellular fluid (VM ECF) acid-base variables.
*Indicates statistical significance when compared with baseline values at time 0.
Po$_2$—oxygen tension; Pco$_2$—carbon dioxide tension; VT—tidal volume; F—respiratory rate. 1kPa = 7·5 mm Hg.

[HCO$_3^-$] was significant. There were no significant changes in the Pco$_2$ or in the H$^+$ or HCO$_3^-$ concentration of the ventral medullary extracellular fluid.

The infusion of aminophylline caused an appreciable rise in ventilation. Ten minutes after the start of the infusion ventilation had risen to 155% of the baseline value (p = 0·003) and there was a further rise at 30 minutes. The increase in ventilation was accounted for by an increase in both frequency of breathing and tidal volume, though the latter was more prominent. Aminophylline caused a small but significant fall in mean arterial blood pressure, mean (SD) values being 126 (20), 117 (23), and 109 (21) mm Hg respectively at time 0 and 10 and 30 minutes after the infusion.

We compared ventral medullary extracellular fluid and arterial blood acid-base variables. The mean values for extracellular fluid Pco$_2$ minus arterial Pco$_2$ were 1·96 (1·07), 1·76 (1·23) and 2·03 (1·09) kPa at time 0 and 10 and 30 minutes after the infusion of aminophylline. These values were not significantly different from each other. The fact that Pco$_2$ was higher than Paco$_2$ in the ventral medullary extracellular fluid accounted for the higher H$^+$ concentration in the extracellular fluid than in the plasma.

About two hours after infusion of aminophylline the mean serum theophylline concentration was 14·2 (SD 1·5) mg/l.

Discussion

The results of this study show that in the anaesthetised spontaneously breathing cat with peripheral chemodenervation the administration of aminophylline, in doses that achieve therapeutic serum concentrations, causes a considerable increase in ventilation with no change in Pco$_2$ or H$^+$ concentration in the ventral medullary extracellular fluid. The pH of the ventral medullary extracellular fluid was slightly more acidic at 30 minutes than at baseline (table) and, although this difference is not statistically significant, it may have contributed to the rise in ventilation to some extent.[7] Ten minutes after aminophylline infusion, however, there was a rise in ventilation without any change in the extracellular fluid pH (table). Conceivably the further rise in ventilation that occurred after 10 minutes was due to ventral medullary extracellular fluid acidosis (table).

In the only previous study[14] to look at this question the pH of the ventral medullary extracellular fluid was measured in anaesthetised, paralysed, and mechanically ventilated cats, and phrenic nerve output measured as an index of ventilation. Theophylline increased phrenic nerve output while the extracellular fluid pH remained constant, in keeping with our data. There are differences in methods between the two studies in that we studied spontaneously breathing

animals and we also measured the Pco$_2$ of the ventral medullary extracellular fluid, so that we were able to show that the rise in ventilation was not due to change in the extracellular fluid Pco$_2$. Although we did not measure cerebral blood flow, the constancy of the extracellular fluid Pco$_2$, and of the difference between this and the arterial Pco$_2$, during 30 minutes of observation indicates that if any change in cerebral blood flow occurred it was matched by an appropriate change in cerebral metabolic rate. We therefore conclude that the ventilatory stimulatory effect of aminophylline occurred in the absence of cerebral vasoconstriction induced acid-base changes in the ventral medullary extracellular fluid.

Thus our data show that in the spontaneously breathing cat aminophylline increased ventilation by a mechanism or mechanisms unconnected with Pco$_2$ and pH. This and other studies[11][12][14][15] in which ventral medullary extracellular fluid acid-base variables and ventilation have been measured simultaneously are important because they dissociate changes in ventilation from known chemical stimuli—that is, the pH and Pco$_2$ of the ventral medullary extracellular fluid. These studies strongly though indirectly suggest that neurotransmitters[4][16][17] are important modulators of ventilation, and that perhaps they interact with known chemical stimuli (Po$_2$, Pco$_2$/H$^+$) to determine the level of ventilation. Recent studies indicate that adenosine and its analogues depress neuronal function[18] and, presumably by the same mechanism, exert an inhibitory action on neurones concerned with the control of ventilation.[4][5][19][20] This action of adenosine is mediated through the extracellular A$_1$ receptor, and is competitively inhibited by theophylline.[5] We have shown[12] previously that hypoxic ventilatory depression is largely prevented by theophylline, suggesting that adenosine may mediate the depression. Inhibition of adenosine with a therapeutic plasma concentration of aminophylline, as reported in the present study, could explain our results. As aminophylline increased baseline resting ventilation considerably we conclude that adenosine, via its central action, normally depresses ventilation tonically and potently.

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