

Effect of aminophylline on the human diaphragm

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ABSTRACT The effect of intravenous aminophylline on the contractile function of the diaphragm was studied in four normal subjects. The contractility of the diaphragm was assessed by the measurement of transdiaphragmatic pressure (Pdi) after right phrenic nerve stimulation at 1 Hz. Pdi was measured before and during aminophylline infusion (6 mg/kg over 30 minutes), during which therapeutic concentrations of theophylline were attained (mean 13.8 mg/l, range 8.5-20.2). The Pdi achieved was not affected by aminophylline. This result suggests that theophylline at therapeutic concentrations has little effect on the contractility of the normal human diaphragm.

Methylxanthines are commonly used to treat patients with airways obstruction and have a beneficial action in relaxing airway smooth muscle and stimulating the central nervous system.¹ These drugs are also known to act on skeletal muscle, producing appreciable potentiation of twitch force in vitro at millimolar concentrations.² It has been suggested that aminophylline in therapeutic dosage may also reduce or reverse respiratory muscle fatigue.³

Muscle fatigue characterised by a selective reduction of force in response to low or moderate frequencies of excitation (less than 30 Hz) with preservation of force at higher frequencies (50-100 Hz) develops in limb muscles with prolonged high work loads.⁴ This low frequency fatigue reflects diminished twitch tension.⁵ With sufficient ventilatory stress low frequency fatigue occurs in the sternomastoid of normal subjects⁶ and patients with airways obstruction⁷ and can also develop in the human diaphragm.^{8,9} In vitro study of strips of skeletal muscle has shown that high concentrations of xanthines potentiate twitch tension sufficiently to reverse low frequency fatigue, whereas concentrations closer to therapeutic levels have little effect.² Several recent in vitro animal studies, however, suggest that aminophylline at therapeutic concentrations may improve the contractile function of the diaphragm.¹⁰⁻¹³ Similarly, in man aminophylline has been reported to increase diaphragm force and to protect the diaphragm from developing low frequency fatigue.³ Using a similar protocol, however, we were unable to show a beneficial effect of

therapeutic concentrations of aminophylline in a limb muscle (adductor pollicis) in man.¹⁴

To clarify whether xanthines at therapeutic concentrations have an effect on the normal human diaphragm we have studied the transdiaphragmatic pressure twitch height in response to phrenic nerve stimulation before and during aminophylline infusions. We chose to study the non-fatigued diaphragm, where the contractile response is constant, because the potentiating effect of aminophylline on twitch tension is known to be as great for fresh as for fatigued skeletal muscle.²

Methods

The subjects were four normal men, aged 30-40 years, with normal respiratory function and no muscle weakness. They were studied relaxed on a couch with the back rest at 45° and with the head and neck supported by firm pillows. The study was approved by the ethical committee of the Brompton Hospital and the subjects gave written informed consent.

Oesophageal pressure (Poes) and gastric pressure (Pg) were measured with two balloon catheters introduced through the nose,¹⁵ latex balloons 10 cm long with a wall thickness of 0.06 mm attached to polyethylene catheters 100 cm long being used. The oesophageal balloon containing 0.4-0.6 ml air,¹⁶ was positioned in the middle third of the oesophagus, about 45 cm from the nares; the gastric balloon, containing 1.5-2.0 ml air (infinite compliance range of balloon 0.2-3.0 ml air), was positioned in the stomach with a distance of 65-70 cm from nares to balloon tip. Each balloon catheter was attached to a differential pressure transducer (Validyne, range \pm

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150 cm water) and measurements were made in relation to atmospheric pressure. Transdiaphragmatic pressure (Pdi) was obtained by electrical subtraction ($P_{di} = P_g - P_{oes}$) and Pdi at resting end expiration was used as a reference zero. To prevent unpleasant sensations due to irritation of the nasal mucosa by the polyethylene tube, a local anaesthetic (xylocaine 2%) was applied to the nasopharynx before each study. The frequency response of the equipment was assessed from the signal generated by a square wave input to the entire apparatus, which rose with a mean half time of 6.3 (SD 1.2) ms, with no detectable phase difference between oesophageal and gastric pressure responses.

The unfiltered evoked muscle action potential of the diaphragm was recorded by means of two lubricated metal surface disc electrodes 1 cm in diameter, placed in the right sixth and seventh intercostal spaces, 3 cm from the costal margin and 2 cm apart.¹⁷ In our experience the recording of the surface electromyogram is as good as intraoesophageal measurements in lean, normal men. The right phrenic nerve was stimulated percutaneously in the supraclavicular region of the neck immediately posterior to the sternomastoid muscle with a bipolar nerve stimulating electrode (Medelec EC 225). The electrodes consisted of felt pads, surface area 30 mm², soaked in saline with 30 mm spacing between contact surfaces. The skin in the supraclavicular fossa was carefully cleaned with spirit. Stimulation was performed by one of the investigators with the test subject relaxed and breathing quietly. The evoked muscle action potential was displayed on an oscilloscope and recorded on paper. It increased in amplitude with increasing voltage up to a plateau at 80–100 volts. During experimental sessions stimulation was performed at 100–150 volts with square wave unidirectional pulses of 50 microsecond duration at about 1 Hz (Digitimer 3072). Small changes in the position of the stimulating electrode altered the evoked muscle action potential and twitch Pdi height, but once the optimal position was found it was possible to maintain this for many minutes with reproducible maximum responses. Voltage was increased until the twitch Pdi height was maximal and increased voltage did not then increase the Pdi response.

Aminophylline was administered via a right forearm vein in a dose 6 mg/kg, 250 mg being given over the first 10 minutes and the remainder over the next 20 minutes. In one subject (No 2), in whom a total dose of 6 mg/kg over 30 minutes repeatedly gave theophylline concentrations at the lower end of the therapeutic range, a further study using 7.5 mg/kg over 30 minutes was performed. After about 10, 20, and 30 minutes of aminophylline infusion venous

blood was sampled from a left forearm vein for estimation of theophylline concentrations. These were measured with an EMIT homozygous enzyme immunoassay (SYVA). During aminophylline infusion heart rate was continuously monitored, full resuscitation equipment was available, and two physicians were in attendance.

To monitor the position of the chest wall and hence the configuration of the diaphragm, two pairs of linearised magnetometers were applied in the midline anteriorly and posteriorly to rib cage and abdomen. One pair was at the level of the fifth intercostal space and the other pair at a point midway between the xiphisternum and the symphysis pubis.

The signals from the evoked muscle action potentials and from the rib cage and abdominal magnetometers and the oesophageal, gastric, and transdiaphragmatic pressures were displayed on a monitor oscilloscope and recorded on a tape recorder (Racal Store 7), and subsequently played back on to a paper recorder (Mingograf) (fig 1).

EXPERIMENTAL PROTOCOL

Throughout each study the subjects maintained the

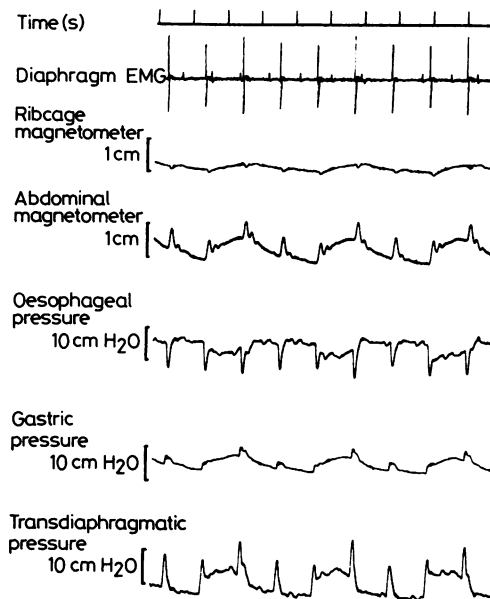


Fig 1 Phrenic nerve stimulation during tidal breathing. Transdiaphragmatic pressure (Pdi) was defined as zero and the baseline magnetometer values were determined during a few seconds' breath holding at resting end expiration. Stimulation for three to five minutes was repeated several times to obtain adequate baseline data. Pdi twitches were analysed only at resting end expiration with constant magnetometer position and only if diaphragm evoked muscle action potential was maximal.

same body position. The maximum phrenic nerve stimulation response in terms of diaphragm EMG and Pdi was established, and the Pdi twitches for several minutes' stimulation at 1 Hz were recorded. The skin of the subject's neck was marked to indicate the optimal position for the stimulating electrodes. The procedure was repeated several times to collect ample control twitches. Aminophylline infusion was then started and the right phrenic nerve was again stimulated for periods of several minutes starting at 10, 20, and 30 minutes. During these periods of stimulation venous blood samples were withdrawn for theophylline estimation.

MEASUREMENTS

The study could not be performed blind because of the symptoms produced by aminophylline given acutely. Electrical stimulation techniques, however, preclude any effect of motivation. To elimination bias during measurement the paper records were reproduced, coded, and analysed without knowledge of which twitches were from the control and which from the aminophylline periods. Twitch Pdi height was measured only at end expiration as indicated by the magnetometer positions and Pg and Poes. The evoked muscle action potential deflection was measured from peak to peak and twitch Pdi height was measured from baseline to peak. Pdi twitches were discarded if they were not at end expiration, were not associated with a maximal evoked muscle action potential, or were marred by pressure artefact—that is, oesophageal contractions. With these strict exclusion criteria 10–20% of twitches were satisfactory. The single maximum twitch height before and during aminophylline infusion was tabulated for each study, and also the mean values from the analysis of all twitches that satisfied the measurement criteria.

Results

An example of the transdiaphragmatic pressure twitch responses before and after aminophylline administered to a therapeutic concentration is shown in figure 2. Aminophylline had no demon-

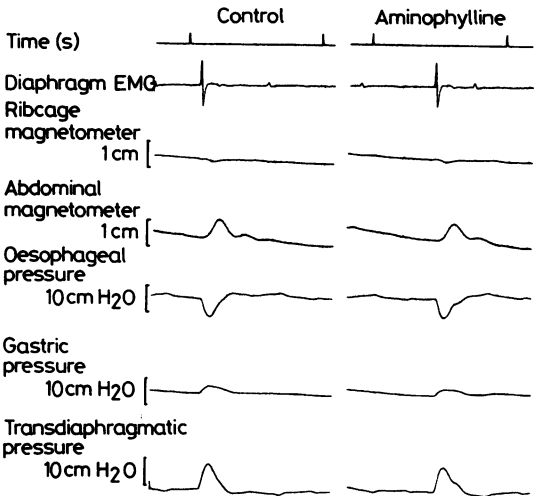


Fig 2 Transdiaphragmatic pressure (Pdi) twitches during tidal breathing before and during aminophylline infusion (theophylline concentration 14.7 mg/l). The twitches are measured at end expiration when the position of the magnetometers is the same for both traces. The Pdi twitch height is not increased by aminophylline.

strable effect on twitch Pdi height. In all four subjects comparison of twitch Pdi height before and during aminophylline infusion showed no significant increase in response during aminophylline (table). Aminophylline raised the heart rate from a mean resting value of 76 to 86 beats per minute, but no cardiac dysrhythmias were noted. Mild nausea was reported by two subjects. One subject (No 2) developed severe nausea when infused with aminophylline at 7.5 mg/kg, so the study was terminated four minutes earlier than planned. On this occasion, despite theophylline concentrations at the upper limit of the therapeutic range (up to 20.2 mg/l) and toxic symptoms, there was no increase in twitch Pdi height.

Discussion

We have found no beneficial action of aminophylline on the twitch Pdi height of the normal human

Transdiaphragmatic pressure (Pdi) twitches before and during aminophylline infusion

Subject No	Pdi (cm H ₂ O)								Theophylline concentration (mg/l)	
	Control				Aminophylline				Mean	Range
	Max	Mean	SEM	n	Max	Mean	SEM	n		
1	8.5	7.3	0.05	54	8.5	7.5	0.07	51	15.2	13.3–16.9
2a	7.0	5.8	0.09	22	8.5	6.0	0.06	117	9.9	8.5–10.9
2b	8.0	6.5	0.09	47	7.5	6.2	0.06	38	19.3	18.8–20.2
3	7.0	5.3	0.11	21	7.5	5.9	0.07	88	10.9	9.3–12.6
4	12.0	10.5	0.07	75	13.5	10.5	0.11	55	13.9	12.5–15.7

diaphragm. In assessing this observation we must give technical factors careful consideration. It is difficult to measure directly the force of contraction of the human diaphragm. Animal studies show that transdiaphragmatic pressure is a good reflection of diaphragm tension.¹⁸ In man the strength of the diaphragm can be assessed by the measurement of Pdi during a maximum inspiratory effort against a closed airway, but the normal range is wide.¹⁹ Furthermore, all voluntary manoeuvres depend on central effort as well as peripheral muscle function. For the study of muscle contractility, nerve stimulation has the advantage of providing a standard excitation independent of volition. Thus the measurement of Pdi in response to phrenic nerve stimulation should provide a good index of diaphragm contractility.

To be confident that an effect of aminophylline on twitch Pdi height has not been obscured by variable excitation the phrenic nerve stimulation was supra-maximal throughout all experiments, increasing voltage producing no change in evoked muscle action potential or twitch Pdi height. Pdi may be importantly affected by lung volume and diaphragm configuration. At lung volumes above functional residual capacity (FRC) the diaphragm is flatter and shorter and a given neural excitation produces less force.²⁰ For these reasons all measurements were made at FRC and at constant diaphragm configuration, as judged by rib cage and abdominal magnetometers.

The measurement of twitch height is a conventional technique of assessing muscle contractility and we therefore chose to study the twitch Pdi height rather than the response to higher stimulation frequencies. In preliminary studies we found that during a single experimental session on a particular subject the twitch height was less variable than the Pdi from phrenic nerve stimulation at higher frequencies.⁸ Experiments on muscle strips show that large concentrations of theophylline maximally potentiate twitch tension, having progressively less effect at high stimulation frequencies, and do not significantly increase tension at 100 Hz.²

There are two possible factors which may influence the twitch Pdi and affect its value for detection of the action of theophylline on diaphragm contractility. Firstly, theophylline could reduce the contraction speed of muscle and prolong relaxation rate, thereby potentiating force at 20 Hz without any detectable increase in twitch height. In vitro studies of limb muscle and diaphragm, however, show that methyl xanthines have little effect on contraction speed,² and for adductor pollicis therapeutic concentrations of theophylline do not affect maximum relaxation rate.¹⁴ Twitch Pdi height could be affected by the frequency response of the measuring

and recording system. The frequency at which the response of the recording system was reduced by 3 decibels (F3dB) was measured and was at least 20 Hz. Any possible influence of frequency response was estimated by inserting a low pass filter into the recorder-display chain. The filter cut off was progressively reduced and the effect on Pdi height measured by superimposing filtered and unfiltered twitch Pdi images on the oscilloscope screen. The F3dB of the low pass filter was reduced to 12 Hz before any reduction in twitch height or shape was seen, which suggests that there were no significant frequency components above 12 Hz. The frequency response of the measuring and recording system is therefore unlikely to have obscured an increase in twitch Pdi.

It is possible that owing to the complexity of studying the human diaphragm a small effect of the drug has not been detected but an important potentiating action is unlikely to have been missed. The large number of twitches recorded and their reproducibility suggest that a small increase of twitch Pdi height should have been detected. On the basis of the standard errors (table) it would be predicted that a change greater than 10% should have been identified.

Jones *et al*² have performed comprehensive studies on strips of animal and human skeletal muscle including diaphragm, and have documented the effects of the methylxanthines (caffeine and theophylline) on both fresh and fatigued muscle. They were able to show that the xanthines greatly potentiated twitch tension and consequently abolished the effects of low frequency fatigue when given in millimolar concentrations. At 0.1 millimolar concentrations, however, they detected no significant effect and concluded that theophylline at therapeutic concentrations would be unlikely to improve muscle contractility, an observation supported by the present study. Several recent in vivo animal studies have suggested that aminophylline does improve diaphragm contractility.¹⁰⁻¹³ Sigrist *et al*¹⁰ studied the effect of aminophylline on the relationship between the diaphragm electromyogram (Edi) and transdiaphragmatic pressure in cats and concluded that for a given Edi aminophylline increases Pdi. Aminophylline was given in high dosage (up to 120 mg/kg), however, and it did not significantly increase the Pdi/Edi ratio until a dose of 20 mg/kg, which corresponded to a theophylline concentration of 22-24 mg/l. On the basis of their data any beneficial effect of aminophylline at a therapeutic blood level (10-20 mg/l) would be small, about 10%.

Aubier *et al*³ performed an important investigation on the action of aminophylline on diaphragm contractility in man. They reported that therapeutic

concentrations of theophylline significantly improved the contractility of the fresh diaphragm and protected the diaphragm from low frequency fatigue, and when low frequency fatigue was experimentally produced aminophylline completely reversed it. The results of this study are thus similar to those of the animal in vitro study of Jones *et al*² but were produced with one tenth the theophylline concentration. Wiles *et al*¹⁴ set out to repeat the experimental protocol of Aubier *et al*, working on the adductor pollicis because force can be recorded with great precision for this muscle. These workers found that at therapeutic theophylline concentrations intravenous aminophylline had no effect on twitch tension or the frequency-force curve and no effect on the development or reversal of low frequency fatigue. Although it is possible that the diaphragm may be more sensitive to the action of aminophylline than adductor pollicis this seems unlikely, since Jones *et al* found that various skeletal muscles responded in the same way when studied in vitro.

There is clearly a discrepancy between the results of our study and much of the previously published work. This paradox is not easily explained. There may be species differences and differences between the sensitivity to aminophylline of different muscles as well as differences in experimental protocols. Further in vivo human studies are required before a final judgement is possible on the magnitude of any effect of aminophylline on the diaphragm.

From the present study we conclude that the diaphragm responds to aminophylline in much the same way as other skeletal muscles. High concentrations that would be toxic clinically may well potentiate twitch tension and reverse low frequency fatigue, but at therapeutic concentrations we found aminophylline to have no substantial direct effect on the contractile properties of the diaphragm of normal subjects.

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