CLINICAL RESEARCH

Parasympathetic nervous system in nocturnal asthma

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Abstract

To investigate the effect of vagal blockade with atropine on nocturnal fall in peak expiratory flow rate 10 patients with asthma who had a diurnal variation in peak expiratory flow rate of >20% were given 30 µg/kg of intravenous atropine or a placebo at 4 am and 4 pm. Vagal blockade caused significant bronchodilatation at 4 am and 4 pm (peak expiratory flow rate rose from 260 to 390 l/min at 4 am and 400 to 440 l/min at 4 pm) and significantly increased the pulse rate from 60 to 121 beats/minute at 4 am and from 76 to 122 beats/minute at 4 pm.

Nocturnal asthma was almost totally reversed, implying that vagal mechanisms are fundamental in its pathophysiology. Other mechanisms—diurnal changes in plasma adrenaline concentration, the activity of non-adrenergic non-cholinergic nerves, and circadian rhythms of inflammatory mediator activity—may also be implicated.

Introduction

Worsening of the symptoms of asthma at night has been recognised for many centuries. Nocturnal asthma occurs in up to two thirds of people with asthma,¹ and most sudden deaths from asthma occur at night.² Hetzel and Clark found that a diurnal variation in peak expiratory flow rate occurred in normal and asthmatic adults, with a peak at about 4 pm and a trough at about 4 am.³ The mean amplitude of the diurnal variation was 51% in adults with asthma and 8% in normal adults. Hetzel and Clark suggested that nocturnal asthma is a consequence of endogenous circadian rhythms acting on the hyperreactive bronchi of people with asthma.

Several circadian rhythms—cortisol, adrenaline concentra-

tions,^{4,6} body temperature,⁷ and sleep^{8,9}—have been studied in relation to nocturnal asthma. Manipulation of the rhythm has been shown to have a significant effect on the diurnal variation in pulmonary function in the case of adrenaline,⁵ body temperature,⁷ and sleep.⁹

Vagal tone increases to the heart at night,¹⁰ and as changes in cardiac vagal activity reflect changes in bronchomotor tone¹¹ this has led to the hypothesis that vagal activity may be important in the pathogenesis of nocturnal asthma. High dose inhaled or nebulised anticholinergic drugs taken at night have been shown to reduce nocturnal asthma.¹²⁻¹⁴ There is dispute, however, whether inhaled anticholinergic drugs alone can inhibit completely the vagal activity to the lungs.^{11,15} Inhaled anticholinergic drugs dilate predominantly central airways whereas drugs administered parenterally have a more uniform effect on the bronchial tree.¹⁶⁻¹⁹

We set out to examine the effects of vagal blockade to the lung by giving intravenous atropine to patients with documented nocturnal asthma at the peak and trough of the circadian rhythm of their pulmonary function.

Method

The 10 asthmatic patients studied had had a diurnal variation in peak expiratory flow rate of more than 20% for more than 75% of the time in the two weeks before the study. Recordings of peak expiratory flow rate at intervals of four hours at home showed that the peak and trough values occurred at about 4 pm and 4 am. The subjects were admitted to hospital, had one day for acclimatisation to the ward routine, and were studied on the next two days. They woke up at 7.30 am, had meals at 8 am, noon, and 5 pm, and went to bed at 10.30 pm. Coffee, tea, and xanthine derivatives had been stopped 48 hours before admission. Inhaled steroids were continued throughout the study. Inhaled β agonists and anticholinergics were not used in the 10 hours before each study session. As it was impossible for the person performing the measurements to be unaware of the side effects of atropine, such as tachycardia and dry mouth, a single blind method was used.

The patients were studied at 4 am and 4 pm on two successive days. An intravenous cannula (Venflon 19 gauge) was inserted at the end of the day of acclimatisation. At 4 am on the first day of the study the sleeping patient was given an intravenous injection of either placebo (sodium chloride 0.9%) or atropine 30 µg/kg. We had found previously that maximal bronchodilatation from vagal blockade was achieved at this dosage: atropine has a peak effect five minutes after injection and this effect persists for at least two hours.¹¹ Thirty minutes after the drug was administered the pulse rate was checked. To check plasma adrenaline concentration 10 ml of blood was taken through the cannula into a precooled lithium heparin tube. The patient was woken

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and three measurements of peak expiratory flow rate made, the best of which was used in subsequent analysis.

At 4 pm on the first day of the study the patient was rested supine for an hour and intravenous placebo or atropine given. The pulse rate, plasma adrenaline concentration, and peak expiratory flow rate were checked 30 minutes later. This protocol was repeated on the second day of the study, varying the drug so that each patient received both placebo and atropine at 4 am and 4 pm. The order of giving the drugs was randomised.

The blood sample was kept in a mixture of ice and water and transferred within 15 minutes to the centrifuge where it was spun for 30 minutes at 4°C and 3000 rpm. The plasma was stored at -70°C until the assay. Adrenaline concentrations were determined by radioenzymatic assay with some modifications to the method of da Prada and Zurcher.²⁰ Samples were assayed in duplicate. The catechol-O-methyltransferase used in the assay was deionised on Sephadex G 25 gel. Enzyme incubation was carried out in disposable polypropylene tubes with 55.5 kBq of tritiated S-adenosylmethionine (555 GBq/mmol, Amersham) for one hour at 37°C. After extraction of the 3-O-methylated products the phases were separated by cooling the tubes in an alcohol bath at -20°C and decanting the organic phase. The metanephrines were freeze dried and the residue was transferred to 20×20 cm Whatman Linear-k preabsorbent thin layer chromatography plates with two 10 µl portions of wash followed by a further 12 µl wash. The spots were removed by suction into plugged Pasteur pipettes, from which they were eluted directly into counting phials. Oxidation of metanephrine and normetanephrine was carried out in the phials for 10 minutes at 37°C, the vanillic acid being extracted into the scintillator and counted without separation of the phases. This method had a lower limit of sensitivity of between 0.05-0.1 nmol for adrenaline, depending on blank values. The intra-assay coefficient of variation was 11% and the interassay coefficient of variation was 16%. Student's *t* test was used for paired comparisons.

Results

Details of the patients are shown in the table. Significant diurnal variation occurred in peak expiratory flow rate ($p<0.01$), plasma adrenaline concentration ($p=0.02$), and pulse rate ($p<0.0001$). Vagal blockade significantly

Physical characteristics of patients (all non-smokers)

Case No	Age (years)	Sex	Duration of asthma (years)	Atopy	Drugs taken	Peak expiratory flow rate (% predicted)
1	30	M	25	+	β Agonists	100
2	50	F	10	+	β Agonists, anticholinergics, inhaled steroids	95
3	45	F	10	-	β Agonists, anticholinergics, inhaled steroids	54
4	32	M	15	+	β Agonists, inhaled steroids	85
5	34	F	25	+	β Agonists	73
6	26	F	20	+	β Agonists	117
7	54	M	15	-	β Agonists, anticholinergics, inhaled steroids, theophyllines	56
8	34	M	30	+	β Agonists, anticholinergics, inhaled steroids	79
9	50	M	2	-	β Agonists, anticholinergics, inhaled steroids	110
10	52	F	6	-	β Agonists, anticholinergics, inhaled steroids, theophyllines	69

increased the pulse rate at both 4 am (121 *v* 60 beats/minute, $p<0.0001$) and 4 pm (122 *v* 76 beats/minute, $p<0.0001$) to levels which were not significantly different from each other (fig 1). Vagal blockade had no effect on plasma adrenaline concentrations (fig 2). Vagal blockade caused significant bronchodilatation at 4 am (peak expiratory flow rate 390 *v* 260 l/min, $p<0.0001$) and at 4 pm (440 *v* 400 l/min, $p=0.04$). The peak expiratory flow rate reached at 4 am after vagal blockade was significantly lower than that reached at 4 pm after vagal blockade (440 *v* 390 l/min, $p<0.01$) but was not significantly different from the value obtained with the placebo at 4 pm (fig 3).

Discussion

The results confirm the findings of diurnal variations in heart rate,¹⁰ peak expiratory flow rate,³ and plasma adrenaline concentrations⁵ in patients with asthma. The diurnal change in heart rate in our subjects could be fully accounted for by changes in vagal tone, as the 4 am and 4 pm values after atropine had been administered were

the same. The diurnal variation in peak expiratory flow rate could largely, but not entirely, be accounted for by changes in vagal activity, implying that other factors also operate.

One such factor is diurnal changes in plasma adrenaline concentration, as postulated by Barnes *et al.*⁵ They infused adrenaline at two concentrations in five asthmatics with nocturnal symptoms and found that an infusion rate of 10 ng/kg/min reversed the fall in peak expiratory flow rate at night. As this infusion rate had been shown to

FIG 1—Pulse rate in patients with asthma given atropine or placebo at 4 am and 4 pm. Rate after atropine is significantly higher at both times ($p<0.0001$).

FIG 2—Plasma adrenaline concentrations in patients given atropine or placebo at 4 am and 4 pm.

produce a plasma concentration of adrenaline at the upper end of the resting daytime physiological range²¹ Barnes *et al* probably overestimated the effects of the change in plasma adrenaline concentration. Morice *et al* described a woman who several years after bilateral adrenalectomy developed asthma with a significant diurnal variation in peak expiratory flow rate,²² and Morrison *et al* showed that correcting the nocturnal decrease in plasma adrenaline concentration after vagal blockade produced no further bronchodilation.²³ These studies suggest that varying concentrations of adrenaline cannot be the primary cause of nocturnal asthma.

FIG 3—Peak expiratory flow rate in patients with asthma given atropine or placebo at 4 am and 4 pm. Rate after atropine is significantly higher than rate after placebo at 4 am ($p < 0.0001$) and 4 pm ($p = 0.04$). Rate after atropine is significantly lower at 4 am than 4 pm ($p < 0.01$).

Adrenaline may, however, inhibit vagal activity via prejunctional receptors,²⁴ and β blockade in people with asthma may cause bronchoconstriction by removing the inhibitory effect of adrenaline on vagal activity^{25, 26}; therefore the only possible role (if any) for adrenaline in nocturnal asthma would be as a modulator of vagal activity.

Another factor that may be important in the control of diurnal variation in bronchomotor tone in asthma is the non-adrenergic non-cholinergic nerves, which are the only neural bronchodilator pathway in humans and which have an inhibitory effect on vagal activity.²⁷ It is possible that these nerves, which run in vagal efferent nerves,²⁸ are affected by mediators from inflammatory cells and thus diminish inhibitory activity on vagal pathways to the bronchi. It is not known whether this activity changes diurnally.

There may be a circadian rhythm in inflammatory mediator activity—for example, histamine⁵ or platelet activation²⁹—but there is no evidence that this is directly responsible for the diurnal variation in pulmonary function in asthma and may simply occur as a secondary effect of other circadian rhythms—catecholamines, for

example. Further research is needed to determine whether the circadian variation of mediator activity is a primary or secondary event in the pathogenesis of nocturnal asthma.

Other factors implicated in the pathogenesis of nocturnal asthma include sleep,⁹ posture,³⁰ gastro-oesophageal reflux,³¹ and body temperature.⁷ It is possible that these factors act through vagal pathways, as has been shown for posture³² and gastro-oesophageal reflux³³ and suggested for sleep.⁹

Possibly the effects of the vagus nerve are enhanced in asthma by hypersensitisation of the muscarinic receptors by inflammatory mediators and that this exaggerates the effects of a normal circadian variation in activity of the vagal nerves. Such a hypothesis ignores the physiological evidence that stimulation of the irritant receptors in airways increases vagal efferent activity to the bronchial tree,³⁴ and it seems reasonable to postulate that inflammation increases reflex activity as well as sensitivity of the end organ. Whichever explanation applies, the result is an increased effect of the vagus nerve on bronchomotor tone at night in asthma.

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