Is nocturnal asthma caused by changes in airway cholinergic activity?

J R CATTERALL, G B RHIND, K F WHYTE, C M SHAPIRO, N J DOUGLAS

From the Department of Respiratory Medicine, City Hospital, Edinburgh

ABSTRACT   A randomised, double blind, placebo controlled crossover trial of high dose nebulised ipratropium was carried out in 10 asthmatic patients with documented nocturnal bronchoconstriction. Patients received nebulised saline or ipratropium 1 mg at 10 pm and 2 am on two nights. Absolute peak flow (PEF) rates were higher throughout the night after the patients had received ipratropium (at 2 am, for example, mean (SEM) PEF was 353 (34) after ipratropium and 285 (29) l/min after placebo). The fall in PEF overnight, however, was similar with ipratropium and placebo. Patients were given a further 1 mg nebulised ipratropium at 6 am on both nights. There was a significant overnight fall in PEF on the ipratropium night even when comparisons were made between the times that maximal cholinergic blockade would be expected, PEF falling between 11.30 pm and 7.30 am from 429 (31) to 369 (40) l/min. The percentage increase in PEF, though not the absolute values, was greater after ipratropium at 6 am than at 10 pm. These results confirm that ipratropium raises PEF throughout the night in asthmatic patients, but suggest that nocturnal bronchoconstriction is not due solely to an increase in airway cholinergic activity at night.

Introduction

Nocturnal bronchoconstriction with an early morning fall in peak expiratory flow (PEF) is common in asthma,[1-3] though the mechanism is poorly understood.[4 5] There is also evidence for an excess of deaths due to asthma at night.[4 6 7]

Neural control of airway calibre is dominated by the activity of cholinergic fibres in the vagus nerve,[8] stimulation of the vagus causing an increase in airway resistance[9 10] and vagal blockade leading to bronchodilatation.[9-11] As cholinergic activity increases at night,[12-14] and as changes in airway calibre during rapid eye movement sleep in dogs are abolished by atropine or by cooling the vagus nerve,[15] we postulated that nocturnal bronchoconstriction in asthma might be due to an increase in cholinergic activity at night. We have studied the effect of airway cholinergic blockade on nocturnal bronchoconstriction in asthma, using large doses of the inhaled anticholinergic drug ipratropium bromide.[16]

Address for correspondence: Dr N J Douglas, Department of Respiratory Medicine, City Hospital, Edinburgh EH10 5SB. (Reprints will not be available.)

Methods

PATIENTS

We studied 10 asthmatic patients (eight of them men) aged 21–51 (mean 40) years. None had had an acute exacerbation of asthma for at least six weeks. All complained of nocturnal wheeze, with a mean circadian change in PEF (measured four times daily for 5–7 days in six patients at the outpatient clinic) averaging 22% (range 13–42%) of the highest daily value. All had reversible airways obstruction with a documented increase in FEV_1 of more than 20% after inhalation of bronchodilators. Seven had positive skinprick test responses to at least two common allergens and seven had a family history of atopy. Three patients were taking prednisolone 5–10 mg daily, eight inhaled corticosteroids, and two inhaled sodium cromoglycate; all this medication was continued unchanged during the study. Other treatments which included oral theophylline (three patients), oral salbutamol (two patients), and inhaled ipratropium bromide (two patients), were withdrawn 24 hours before the study. All patients were receiving inhaled $beta_2$ agonists, which were withdrawn six hours before the study. Subjects gave informed consent to the study, which was approved by the local ethics committee.
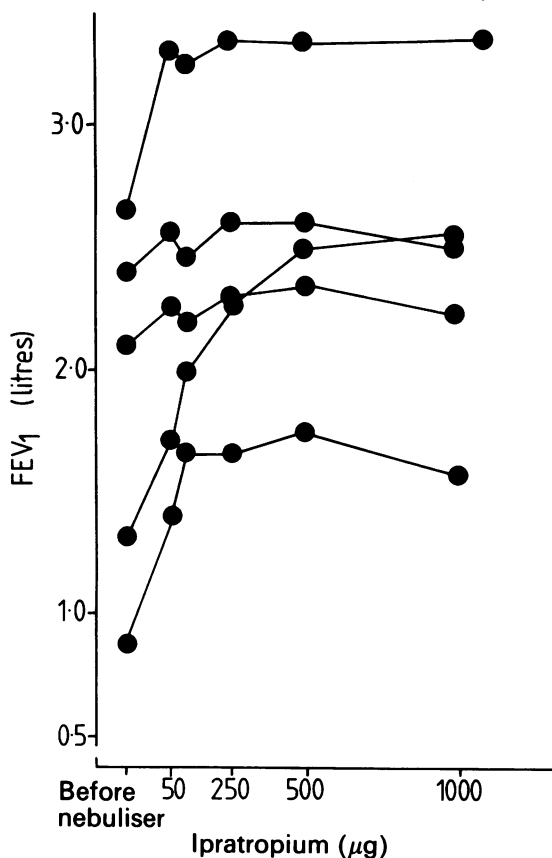
Fig 1 *Effect of inhaled ipratropium on FEV$_1$ in five patients with asthma: cumulative dose-response curve.*

Fig 2 *Mean (SEM) overnight changes in peak expiratory flow in asthmatic patients on the night when they inhaled ipratropium (I, ●) and the night when they inhaled placebo (P, ○) (n = 10 for the first three time points and nine for the rest). Ipratropium (1 mg) or placebo was inhaled at 10 pm and 2 am, ipratropium (1 mg) at 6 am, and terbutaline (B) (5 mg) at 7.30 am.*

IPRATROPIUM DOSE-RESPONSE CURVES
One week before the night time study cumulative dose-response curves for ipratropium were obtained mid morning from five of the patients, FEV$_1$ being measured after inhalation of 50, 250, 500, and 1000 μg of the drug, administered from an Acorn nebuliser at a flow rate of 8 l/min. As maximal bronchodilatation occurred in all patients with 250–500 μg (fig 1), a dose of 1 mg was chosen for the study.

STUDY DESIGN
The patients were studied on three consecutive nights, the first being for acclimatisation. On the second and third nights they received nebulised ipratropium (1 mg) or nebulised normal saline (placebo) in random order and double blind (fig 2). They arrived in the laboratory at 9.30 pm. At 10 pm PEF was measured and nebulised normal saline or ipratropium 1 mg was administered. Ninety minutes later—the time when the maximum response to ipratropium would be
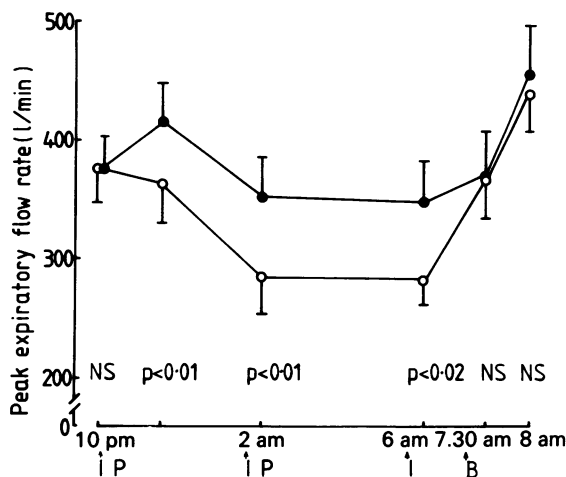
expected[16][17]—PEF was measured again. The patients were allowed to sleep, but were wakened at 2 am, when PEF was again recorded and a further dose of placebo or ipratropium 1 mg was given by nebuliser. They were woken again at 6 am for a PEF measurement, followed on both nights by nebulised ipratropium 1 mg. PEF was measured again 90 minutes later and on both nights nebulised terbutaline 5 mg was given and a final PEF measurement made 30 minutes later (fig 2). At 10 pm, 11.30 pm, 2 am, and 6 am the heart rate (radial pulse) was measured after the patient had woken for the PEF measurements and administration of the nebulised solutions.

Four patients were studied again on a further two nights, receiving ipratropium 1 mg or nebulised saline at 10 pm and 2 am according to a double blind, random order protocol. At 6 am they underwent a methacholine inhalation challenge.[18] The patients inhaled saline followed by doubling concentrations of methacholine from 0·025 μg/ml during tidal breathing for two minutes. The aerosol was generated by oxygen at a flow rate of 8 l/min from an Acorn nebuliser. FEV$_1$ was measured 30 and 90 seconds after the end of the inhalation, and the study was discontinued when the FEV$_1$ fell by 20% or more from the post-saline value. The concentration of methacholine that produced a 20% fall in FEV$_1$ (PC$_{20}$) was obtained by linear interpolation from the log dose-response curve.

ANALYSIS
Values are quoted as means with standard errors in

Peak expiratory flow rates after placebo and ipratropium

	Mean (SEM) peak flow (l/min)				
	10 pm	11.30 pm	2 am	6 am	7.30 am
Placebo	376 (29)	363 (33)	353 (34)	283 (21)	366 (32)
Ipratropium	376 (31)	415 (33)	285 (29)	347 (37)	369 (40)
n*	10	10	10	9	9
p	NS	<0·01	<0·01	<0·02	NS

*Number of patients with measurements for analysis.

parentheses. Differences between results were assessed by the paired t test with a Bonferroni correction for multiple comparisons when appropriate.[19]

Results

Baseline PEF values at 10 pm were similar on the ipratropium and placebo nights (table, fig 2). One patient woke with wheeze between 1 and 2 am on both study nights and required a bronchodilator. His

results up to this time only were included in the analysis.

At 10 pm PEF rose after ipratropium but not after placebo and the bronchodilating effect of ipratropium persisted throughout the night (table, fig 2). The overnight fall in PEF (PEF at 11.30 pm − PEF at 6 am) did not differ significantly, however, between the ipratropium and placebo nights (mean (SEM) ΔPEF = 80 (21) v 96 (22) l/min), nor was there a difference between this fall with placebo and that occurring by 7.30 am after the ipratropium night (60 (19) l/min; p > 0·2). There was also no difference in the overnight fall in PEF on the two nights if this was calculated as the fall from 11.30 pm values to the lowest subsequent PEF value (ΔPEF = 103 (23) for placebo and 86 (17) l/min for ipratropium; p > 0·2). Nor did the percentage fall in PEF overnight differ between the ipratropium and placebo nights when the results were analysed in either of the above two ways (p > 0·2 and >0·9 respectively).
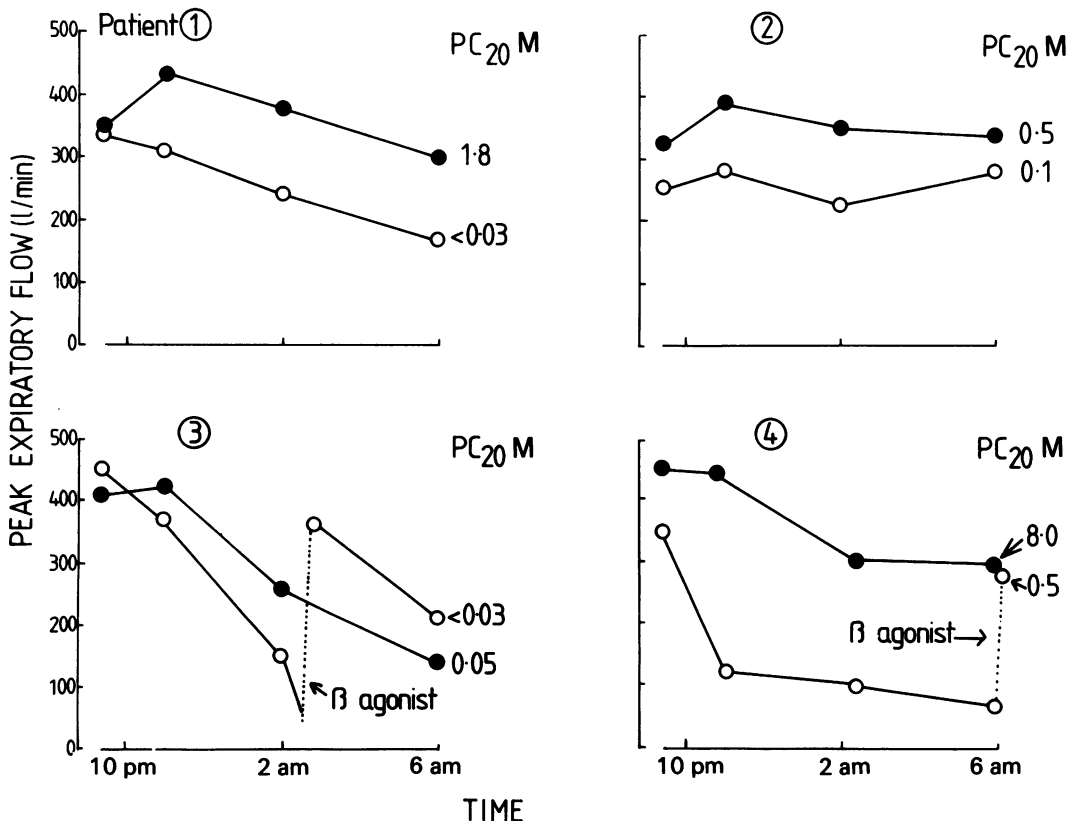
The absolute increase in PEF after ipratropium at

Fig 3 *Overnight changes in peak expiratory flow rates and the provocative concentrations of methacholine causing a 20% fall in FEV_1 (PC_{20}) at 6 am in four asthmatic patients on the night when they inhaled ipratropium (●——●) and on the night when they inhaled placebo (○——○). Patients 3 and 4 were also given inhaled terbutaline to relieve bronchoconstriction on the placebo night at the times indicated by the dotted lines (see text).*

10 pm and 6 am did not differ significantly (p > 0·05); but because baseline PEF values were different the percentage increase in PEF produced by ipratropium was greater at 6 am (p < 0·02). All nine patients showed a further rise in PEF after terbutaline at 7.30 am on both days (fig 2, p < 0·0001). The PC_{20} methacholine at 6 am was higher after ipratropium than after placebo in the four patients who underwent methacholine challenge (fig 3), though two of the patients had received inhaled terbutaline (4 mg at 2.30 am, 500 mg at 6 am) before methacholine on the placebo night because the FEV_1 was below 1 litre.[20]

The mean heart rate at 10 pm (ipratropium 76 (SEM 3), placebo 72 (3) beats/min) did not differ significantly from the heart rate at 11.30 pm, 2 am, or 6 am on either night (ipratropium 69 (4), 64 (4), 67 (6); placebo 69 (4), 70 (4), 75 (4)).

Discussion

The purpose of this study was to determine whether nocturnal bronchoconstriction in asthma is due to increased cholinergic activity at night. The results indicate that although cholinergic factors may play a part—as suggested by the greater percentage increase in PEF produced by ipratropium at 6 am than at 10 pm—this is not the main cause. Bronchoconstriction that was not blocked by ipratropium was demonstrated more readily. Ipratropium did,. however, increase PEF throughout the night in these patients and may be a useful form of treatment for nocturnal asthma.

If cholinergic factors had been a dominant cause of nocturnal asthma, we would have expected less overnight bronchoconstriction after ipratropium than after placebo. This was not observed, whether we compared the overnight fall in PEF between 11.30 pm and 6 am or between the 11.30 pm value and the lowest PEF value. The latter analysis requires evidence that cholinergic blockade was achieved throughout the night, and the results of the methacholine inhalation challenge at 6 am suggest that this occurred. Unfortunately two of the patients required a $beta_2$ agonist on the placebo nights before methacholine challenge, but the acute administration of $beta_2$ agonists would be expected to decrease the response to inhaled methacholine,[21] whereas on both occasions the patients were more responsive on the placebo night after their $beta_2$ agonist than they were on the ipratropium night.

The differences in bronchodilatation induced by ipratropium at 10 pm and 6 am are difficult to interpret because baseline airway calibre was different at the two times. The greater increase in percentage change in PEF at 6 am is compatible with there being greater vagal activity in the morning, as a result either of an increase in cholinergic discharge from vagal efferent nerves or of an increased sensitivity of the bronchial smooth muscle to acetylcholine. The observation that cholinergic effects on the cardiovascular system are also increased during the night[14] would be in keeping with increased discharge of acetylcholine as the vagus nerve provides the parasympathetic innervation to both systems. Reinberg et al [22] showed an increase in airway reactivity to inhaled acetylcholine in the early morning but their observation is difficult to interpret because they also showed a synchronous narrowing of the airways, which would itself tend to increase bronchial reactivity. Thus we cannot say whether the greater percentage change in PEF induced by ipratropium at 6 am than at 10 pm was due to a change in cholinergic discharge, in baseline airway calibre, or in bronchial reactivity to acetylcholine.

Gaultier and colleagues[13] showed that ipratropium had greater effects on lung resistance at 8 am than at 11 pm and that ipratropium abolished circadian changes in dynamic compliance. Measurements were made 10 minutes after ipratropium, however, whereas the peak effects occur after at least one hour[16 17 23] and the clinical significance of changes in dynamic compliance is not clear.[24] Other suggestions that increased vagal tone at night might contribute to nocturnal bronchoconstriction have been based on more circumstantial evidence. Urinary cyclic guanosine monophosphate (cGMP) levels increase at night in asthmatic children,[25] and Reinhardt and colleagues suggested that this may reflect increased vagal tone at night. The relation between cGMP levels and vagal tone, however, is uncertain. Soutar and colleagues[26] postulated an increase in vagal tone at night to explain their observation that nocturnal peak flow changes paralleled heart rate changes in three of seven asthmatic patients, and vagal factors were also implicated by Postma and colleagues,[14] who found that eight patients with chronic airflow limitation and nocturnal bronchoconstriction had slower heart rates and longer sinus arrthythmia gaps than healthy control subjects, the differences being maximal at night. Although the results of all these studies are consistent with an increase in cholinergic activity at night, at least in the heart, they do not provide proof, nor do they establish a causal relation between cholinergic activity and nocturnal bronchoconstriction.

Despite bronchodilatation with ipratropium at 7.30 am with doses that produced maximal bronchodilatation during the day, inhaled beta agonists produced an additional rise in PEF. This suggests that important non-cholinergic factors are operating at this time.

The above apportioning of cholinergic and non-cholinergic factors depends on ipratropium blocking

muscarinic receptors in the airway smooth muscle without having any other effect on airway calibre. Different types of muscarinic receptors have recently been recognised, and one of these (the prejunctional postganglionic muscarinic receptor), when inhibited by atropine like drugs, might promote acetylcholine release.[27] Further studies are required to ascertain the role of vagal activity and of different cholinergic receptors in the pathogenesis of nocturnal asthma.

Although ipratropium did not alter the magnitude of the "nocturnal dip" in these patients, it did increase overnight peak flow. As it is a relatively long acting preparation it may be useful in nocturnal asthma, as suggested by two recent clinical studies of inhaled anticholinergic drugs.[28] [29]

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