

The effect of anticholinergic and beta-agonist pretreatment on bronchoconstriction induced by N-Formyl-methionyl-leucyl-phenylalanine

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ABSTRACT: N-Formyl-methionyl-leucyl-phenylalanine (FMLP), an acylated tripeptide bacterial neutrophil chemotactic factor, has been shown to be a bronchoconstrictor by inhalation in man *in vivo*. It thus has a putative role in the generation of bronchoconstriction associated with bacterial bronchial infection. We have investigated the effect of pretreatment with the anticholinergic agent, ipratropium bromide (IB), and the beta₂-agonist, fenoterol (F), on FMLP-induced bronchoconstriction. Ten non-asthmatic subjects aged 21-28 yrs performed dose-response curves to nebulized FMLP on 3 study days after pretreatment with saline, F or IB. Amongst the 8 subjects who bronchoconstricted by 20% to FMLP there was a significant increase in the provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV₁) (PC₂₀FMLP) after both IB and F. F was significantly better than IB. When comparison was made using absolute fall in FEV₁ and including all 10 subjects the same results were found. Partial inhibition of FMLP-induced bronchoconstriction by IB suggests that part of the effect of FMLP is vagally mediated. We suggest that F is acting *via* modulation of FMLP-induced rises in intracellular free calcium.

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Bronchial infection is the most common precipitant of acute respiratory failure in chronic obstructive pulmonary disease (COPD) [1, 2]. Traditional teaching is that bronchoconstriction associated with bacterial bronchial infection in patients with chronic airflow limitation is a consequence of a combination of mucosal oedema and mucus plugging. Interest in the possibility that there is an additional specific bacterial mediator of bronchial smooth muscle contraction has led investigators to look at the potential of endotoxin produced by *Haemophilus influenzae* to produce bronchoconstriction [3] or enhanced alpha-adrenergic responsiveness [4] and to look for histamine release by bacteria [5]. However, we have proposed that another factor may be of importance in this clinical situation. N-Formyl-methionyl-leucyl-phenylalanine (FMLP) is a potent neutrophil chemotactic factor [6]. Virtually all bacteria [7] produce low molecular weight neutrophil chemotactic factors which are known to be oligopeptides [8]. Previous work has shown FMLP to contract human bronchial smooth muscle *in vitro* [9] and to be a bronchoconstrictor when given by inhalation in man *in vivo* [10]. Although bronchial smooth muscle contraction by FMLP was not inhibited by atropine, vagal reflexes may

contribute to the bronchoconstriction *in vivo* as one of the effects of FMLP is an irritating cough.

The aim of this study was to determine and compare the extent to which bronchoconstriction induced by FMLP could be blocked by pretreatment with an adrenergic beta₂-agonist or an anticholinergic agent.

Methods

Eleven subjects recruited from the staff and students of Concord Hospital gave informed consent to participate in this study. Subjects were selected on the basis of no previous history of asthma or other respiratory disease. The protocol used had been approved by the Human Ethics Review Committee of Concord Hospital. All subjects were current nonsmokers. One was an ex-smoker who had smoked a total of 5 pack-years. No subject was on bronchodilator or other drug therapy. No subject was challenged with FMLP within 3 weeks of a viral or bacterial respiratory tract infection.

FMLP (Sigma Chemical Company, St Louis, Missouri) was dissolved in 50% dimethylsulphoxide

(DMSO) in saline. Solutions containing doubling concentrations of FMLP from $0.12 \text{ mg}\cdot\text{ml}^{-1}$ to $4 \text{ mg}\cdot\text{ml}^{-1}$ were freshly prepared on each study day. The DMSO/saline mixture was taken as the control. The solutions for pretreatment were saline 2 ml, fenoterol 1 mg/saline 1 ml and ipratropium bromide 0.5 mg (2 ml). The ipratropium bromide was an isotonic preparation containing the preservatives edetic acid and benzalkonium chloride.

Spirometry was performed at baseline using a Vitalograph spirometer and forced expiratory volume in one second (FEV_1) was recorded. The appropriate pretreatment solution was then nebulized for 5 minutes using air at $8 \text{ l}\cdot\text{min}^{-1}$ and a Hudson nebulizer. After fenoterol and saline, FEV_1 was recorded at 5 minute intervals for 15 minutes to determine the bronchodilator response to pretreatment. After ipratropium bromide because of the slower onset of action [11], FEV_1 was recorded at 15 minute intervals for 45 minutes. Bronchodilator response was expressed as the percentage change in FEV_1 at the third post-treatment point compared to baseline.

Immediately thereafter a standard doubling-dose response curve to FMLP was performed. The initial concentration given was $0.12 \text{ mg}\cdot\text{ml}^{-1}$ as no subject had bronchoconstricted by 20% to this concentration in a previous study [10]. The subject took 5 breaths from functional residual capacity (FRC) to total lung capacity (TLC) of each concentration of FMLP using a de Vilbiss 646 nebulizer and a French-Rosenthal dosimeter. FEV_1 was repeated after 3 minutes and subsequent doubling concentrations of FMLP were given until a 20% fall in FEV_1 was seen or the highest concentration of $4 \text{ mg}\cdot\text{ml}^{-1}$ had been given. Dose response curves using FEV_1 were constructed and the provocative concentration causing a 20% fall in FEV_1 ($\text{PC}_{20}\text{FMLP}$) was determined by linear interpolation of the log-dose response curve.

In each case the first study day was with saline pretreatment and the pretreatment order for the remaining days was randomized. Any subject not responding with a 10% fall in FEV_1 to FMLP on the saline day was excluded. Subjects responding with a fall in FEV_1 of between 10 and 20% were included only in the comparison of absolute changes and not $\text{PC}_{20}\text{FMLP}$.

Some subjects responded by 20% to the initial concentration of FMLP, $0.12 \text{ mg}\cdot\text{ml}^{-1}$. To allow a statistical analysis, their PC_{20} was recorded as $0.12 \text{ mg}\cdot\text{ml}^{-1}$. Similarly, some subjects after treatment had not responded at the highest concentration given. These were recorded as a $\text{PC}_{20}\text{FMLP}$ of $4 \text{ mg}\cdot\text{ml}^{-1}$. The dose response curves on any study day terminated after a 20% fall in FEV_1 was reached. Therefore the final doses given to a subject on different study days were non-identical. In order to allow a comparison of the effect of pretreatment based on change in FEV_1 , comparison between pretreatments was made at the largest concentration common to all 3 study days.

All data was treated as non-parametric and comparison of the log-transformed $\text{PC}_{20}\text{FMLP}$ and percent fall in FEV_1 following each pretreatment was made using

the Wilcoxon signed ranks test. A value of $p < 0.05$ was regarded as significant.

Results

Eleven subjects aged 21–28 yrs commenced the study. All had baseline lung function in the normal range. One subject failed to respond to FMLP with a 10% fall in FEV_1 and was excluded. Mean bronchodilator response $\pm \text{SEM}$ after saline was $1.3 \pm 0.6\%$, after fenoterol $3.4 \pm 1.1\%$ (NS) and after ipratropium bromide $4.3 \pm 1.0\%$ (NS). Effects other than bronchoconstriction noted after FMLP included facial flushing in 3 subjects and cough in 8. Facial flushing was unaffected by pretreatment with either fenoterol or ipratropium bromide. No other effect of FMLP was noted.

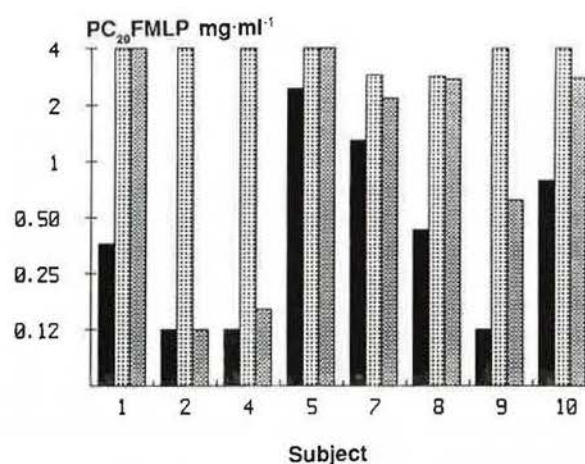


Fig. 1. – Provocative concentration of N-formyl-methionyl-leucyl-phenylalanine (FMLP) causing a 20% fall in forced expiratory volume in one second (FEV_1) ($\text{PC}_{20}\text{FMLP}$) for each subject following pretreatment with saline, fenoterol and ipratropium. Subjects 3 and 6 did not respond to FMLP with a 20% fall in FEV_1 . Saline: ■; fenoterol: ▨; ipratropium bromide: ▩.

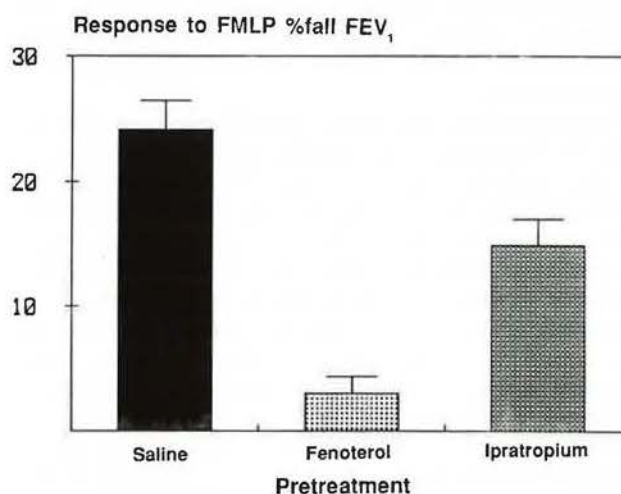


Fig. 2. – Mean percentage fall in FEV_1 in response to FMLP, after each pretreatment. Comparison was made at the last dose which was common to all 3 days for each subject. Error bars indicate standard error. For abbreviations see figure 1.

Eight of the 10 subjects had a 20% fall in FEV_1 after saline and were suitable for comparison based on $PC_{20}FMLP$. The geometric mean $PC_{20}FMLP$ (95% confidence limits) following saline pretreatment was $0.40 \text{ mg}\cdot\text{ml}^{-1}$ (0.18, 0.88), after fenoterol $3.67 \text{ mg}\cdot\text{ml}^{-1}$ (1.40, 4.08) and after ipratropium bromide $1.16 \text{ mg}\cdot\text{ml}^{-1}$ (0.44, 3.02). $PC_{20}FMLP$ after fenoterol was greater than after saline ($p < 0.01$) and ipratropium bromide ($p < 0.02$). $PC_{20}FMLP$ after ipratropium bromide was also significantly greater than after saline ($p < 0.05$) (fig. 1).

In the comparison based on percentage change in FEV_1 , the fall in FEV_1 after saline was $24.2 \pm 2.3\%$, after fenoterol $3.0 \pm 1.3\%$ and after ipratropium bromide $14.9 \pm 2.1\%$. Response after fenoterol was again less than after saline ($p < 0.01$) and ipratropium bromide ($p < 0.01$). There was also a significantly smaller fall in FEV_1 after ipratropium bromide than saline ($p < 0.01$) (fig. 2).

Discussion

Neutrophil infiltration is a feature of the airway inflammation in asthma and bacterial bronchial infection. It is known that a number of products of activated neutrophils including prostaglandins and leukotrienes are active as bronchoconstrictors. Agents such as FMLP and platelet activating factor, which are pro-inflammatory and also independently contract bronchial smooth muscles are useful probes into the relationship between neutrophils, neutrophil activation and bronchoconstriction. FMLP is peculiar in that it is produced by the bacteria commonly associated with bronchial infection and may thus be of importance in bronchoconstriction in this particular setting.

In this study we have found that pretreatment with either the beta-agonist, fenoterol, or the anticholinergic agent, ipratropium bromide, significantly inhibits bronchoconstriction induced by FMLP inhalation. Fenoterol increased the $PC_{20}FMLP$ by approximately 3 doubling dilutions and was significantly more effective than ipratropium bromide, which increased $PC_{20}FMLP$ by 1.5 doubling dilutions. Comparisons based on FMLP-induced fall in FEV_1 after each pretreatment yielded similar results.

Superior protection against induced bronchoconstriction by beta-agonists in comparison to anticholinergics is also seen after histamine and exercise challenge in asthmatics, as reviewed by TATTERSFIELD [12]. In asthmatics this occurs despite similar bronchodilator responses to each drug, suggesting that the drugs have different modes of action which are not limited to the geometric effect of pre-challenge bronchodilatation. In this study the subjects had normal lung function at baseline and only exhibited a minimal bronchodilator response to both fenoterol and ipratropium bromide.

Protection offered by the anticholinergic agent ipratropium bromide against bronchoconstriction induced by FMLP *in vivo* contrasts with the ineffectiveness of atropine *in vitro* [9]. The bronchial smooth muscle strips were obtained from older ex-smokers having lung resections and are representative of a different subject

population from those in this study. However, this is unlikely to totally account for the conflicting observations. It is more likely that partial inhibition by ipratropium bromide *in vivo* reflects a contribution of vagal reflexes to the bronchoconstriction. Our observation of pronounced cough following FMLP inhalation would be in keeping with an effect on bronchial irritant receptors with consequent bronchoconstriction. Although a presynaptic action of FMLP on vagal efferents is possible, there is no evidence for such action in any animal species thus far studied.

Preparations of ipratropium bromide containing the preservatives edetic acid and benzalkonium chloride have been implicated in the paradoxical bronchoconstriction seen in some asthmatic subjects with this agent [13]. It is possible that the apparent greater efficacy of fenoterol over ipratropium bromide is related to this phenomenon. However, this has only been reported in asthmatics and all subjects in this study were non-asthmatic. Furthermore no subject showed bronchoconstriction after inhalation of ipratropium bromide.

Bronchial smooth muscle contracts after activation of the actino-myosin complex by increased intracellular free calcium. This increase can come from influx through voltage dependent or receptor-mediated membrane channels or from intracellular stores [14]. It is known that FMLP increases intracellular calcium levels in human tracheal smooth muscle *in vitro* and that restriction of extracellular calcium reduces the maximal contractile response to FMLP [15]. Beta-agonists such as fenoterol increase intracellular cyclic-AMP levels, relaxing smooth muscle by enhancement of calcium reuptake [16]. It is most likely that fenoterol inhibits FMLP-induced bronchoconstriction by this mechanism.

In conclusion, we have found significant partial inhibition by ipratropium bromide of FMLP-induced bronchoconstriction in man *in vivo*. This suggests a role for vagal reflexes in the genesis of the observed bronchoconstriction. Fenoterol was significantly more effective than ipratropium bromide virtually abolishing bronchoconstriction after FMLP. We speculate that this is because of modulation of FMLP-induced increases in intracellular calcium concentrations.

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Effet d'un pré-traitement anticholinergique et bêta-agoniste sur la bronchoconstriction induite par la N-formyl-méthionyl-leucyl-phenylalanine. M. Peters, A. Breslin, N. Berend.

RÉSUMÉ: La N-formyl-méthionyl-leucyl-phenylalanine (FMLP), tripeptide bactérien acétylé, facteur chemotactique pour les neutrophiles, s'est avérée bronchoconstrictrice en inhalation chez l'homme *in vivo*. Elle pourrait donc avoir un rôle dans la genèse de la bronchoconstriction associée à l'infection bactérienne bronchique. Nous avons investigué l'effet d'un pré-traitement par un agent anticholinergique, le bromide d'ipratropium (IB) et par le bêta-2-agoniste, fenoterol (F), sur la bronchoconstriction induite par FMLP. Dix sujets non asthmatiques âgés de 21 à 28 ans ont exécuté des courbes dose-réponse à la nébulisation de FMLP à trois jours d'étude respectivement après pré-traitement par la solution saline, le fenoterol, ou le bromide d'ipratropium. Parmi les 8 sujets qui ont eu une bronchoconstriction de 20% après FMLP, il y avait une augmentation significative du PC₂₀ FMLP à la fois après IB et F. F était significativement supérieur à IB. Quand la comparaison est calculée en utilisant la chute du VEMS en valeur absolue et en incluant les dix sujets, les mêmes données sont retrouvées. Une inhibition partielle de la bronchoconstriction induite par FMLP grâce à IB, suggère qu'une partie de l'effet de la FMLP est à médiation vagale. Nous suggérons que F agit par modulation des augmentations de calcium libre intracellulaire induites par la FMLP. *Eur Respir J.*, 1989, 2, 946–949.