The effects of β_2 -agonists and methylxanthines on neutrophil function *in vitro*

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The effects of β_2 -agonists and methylxanthines on neutrophil function in vitro. C.G. Llewellyn-Jones, R.A. Stockley. ©ERS Journals Ltd 1994.

ABSTRACT: Therapeutic agents which affect polymorphonuclear neutrophil (PMN) functions have the potential to reduce or increase PMN activation and, hence, influence the progression of lung inflammation.

We have assessed the effects of the β_2 -agonist, terbutaline, and the methylxanthine, aminophylline, on PMN functions *in vitro* at both therapeutic and higher concentrations.

At therapeutic levels, both agents increased PMN chemotaxis to formyl-methionyl-leucyl-phenylalanine (FMLP) in a dose-dependent manner from a control value of 22.5 ± 3.58 cells-field⁻¹ to 26.1 ± 4.73 cells-field⁻¹ with $4 \text{ mg} \cdot \text{l}^{-1}$ terbutaline, and to 26.3 ± 4.49 cells-field⁻¹ with $20 \text{ mg} \cdot \text{l}^{-1}$ aminophylline. When the cells were preincubated with higher doses of the agents in separate experiments there was inhibition of chemotaxis from a control value of 31.1 ± 2.06 cells-field⁻¹ to 18.3 ± 0.82 cells-field⁻¹ at $160 \text{ mg} \cdot \text{l}^{-1}$ terbutaline, and to 16.1 ± 0.77 cells-field⁻¹ at $400 \text{ mg} \cdot \text{l}^{-1}$ aminophylline. A similar effect was seen when the PMNs were preincubated with terbutaline and aminophylline prior to assessment of superoxide anion generation, with stimulation of superoxide release at therapeutic levels of the drugs and inhibition at higher doses (19% increase from resting control cells at terbutaline $4 \text{ mg} \cdot \text{l}^{-1}$ and 53% reduction at $160 \text{ mg} \cdot \text{l}^{-1}$; 28% increase with aminophylline $20 \text{ mg} \cdot \text{l}^{-1}$ and 22% reduction at $400 \text{ mg} \cdot \text{l}^{-1}$). Both terbutaline and aminophylline had no effect on PMN degranulation, as assessed by the degradation of fibronectin.

These data suggest that terbutaline and aminophylline exert a biphasic effect on PMN functions *in vitro*, and may have detrimental effects on lung tissues at therapeutic levels through potentiation of PMN recruitment and activation. However, the observed effects of therapeutic doses of these agents on *in vitro* PMN functions were relatively small and, therefore, the clinical relevance of these results is, as yet, uncertain.

Eur Respir J., 1994, 7, 1460–1466.

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Keywords: β_2 -agonists
methylxanthines
neutrophils

Received: September 21 1993
Accepted for publication May 5 1994

Polymorphonuclear neutrophils (PMNs) play a major role in host defence against invading micro-organisms. However, PMNs have also been implicated in the pathogenesis of several inflammatory diseases, including chronic destructive lung diseases, through recruitment to the lungs and the release of proteinases and oxygen metabolites within lung tissues. Studies of PMNs isolated from the peripheral blood of patients with emphysema have shown an increased chemotactic response to formyl-methionyl-leucyl-phenylalanine (FMLP) compared to PMNs isolated from control subjects [1]. These results suggest that, for a given chemotactic signal, larger numbers of PMNs than normal would be recruited to the lungs of patients with emphysema. This is consistent with bronchoalveolar lavage studies of patients with chronic bronchitis, which have shown increased numbers of PMNs in the lungs compared to healthy control smokers [2]. In addition, the circulating PMNs from patients with emphysema were also more active at digesting connective

tissues by degranulation than PMNs from healthy control subjects [1]. These results would suggest that each PMN recruited to the lungs of a patient with emphysema would be capable of causing more tissue damage than normal. These two factors may play a key role in the pathogenesis of emphysema [1].

Therapeutic agents which inhibit PMN functions may prove useful in reducing the progression of chronic destructive lung diseases, but agents which potentiate PMN function may lead to increased inflammation, tissue damage and, hence, progression of the disease. Patients with chronic bronchitis and emphysema are sometimes prescribed bronchodilators, either β_2 -agonists, methylxanthines or both agents in combination, often without clear evidence of reversible airways disease. The effect of these agents on PMN function is unclear, with some studies reporting inhibition of PMN functions *in vitro* [3–5], whilst others have reported potentiation of PMN functions [6, 7]. However, many of the studies reporting

inhibition of PMN function by methylxanthines and catecholamines *in vitro* were performed at doses higher than the accepted therapeutic levels. The effect of these agents on PMN function at therapeutic levels, thus, remains unclear, although two studies have suggested that methylxanthines may have a biphasic effect on PMN function, with potentiation at therapeutic levels and inhibition at higher concentrations [6, 7].

As a result of the current controversies in the literature, the present study was designed to evaluate the potential influence of methylxanthines and β_2 -agonists on disease progression in patients with chronic bronchitis and emphysema, who are treated with these agents. We assessed the effects of aminophylline and terbutaline, firstly at therapeutic concentrations and secondly at concentrations above the therapeutic ranges, in order to determine whether these agents do have a biphasic effect on chemotaxis, superoxide anion generation and degradation of connective tissues by PMN isolated from normal healthy controls.

Methods

Isolation of blood neutrophils

PMNs were isolated using the method of JEPSEN and SKOTTUN [8]. Briefly, venous blood from normal healthy controls was collected into lithium heparin tubes. Each sample was diluted with an equal volume of 0.15 M sodium chloride and layered onto a Percoll gradient (Sigma Chemicals, Dorset, UK). The top layer consisted of 2 ml of 54% Percoll (density 1.075 g·ml⁻¹) and the lower layer 3 ml of 78% Percoll (density 1.096 g·ml⁻¹). The tubes were centrifuged for 25 min at 200×g at room temperature. The PMNs (>96% pure, >98% viable, by exclusion of trypan blue) were harvested from the interface of the 54% and 78% layers, washed twice in 0.15 M saline solution, counted and resuspended at the required concentration in relevant assay medium: RPMI 1640 medium (Flow Laboratories, Rickmansworth, UK) for the fibronectin degradation assay; RPMI solution containing 2 mg·ml⁻¹ bovine serum albumin for the chemotaxis assay; or phosphate buffered saline (0.16 mol·l⁻¹, pH 7.2) containing 1 mmol·l⁻¹ calcium chloride and 1 mmol·l⁻¹ magnesium chloride for the superoxide assay. All reagents were confirmed to contain less than 20 ng·l⁻¹ of endotoxin activity using the KabiVitrum Coatest (Flow Laboratories, Rickmansworth, UK).

PMN chemotaxis

The chemotaxis assay was based on the method described by FALK *et al.* [9], using the 48-well microchemotaxis chamber. The lower wells contained 27 μ l of the chemoattractant 10⁻⁸ mol·l⁻¹ FMLP in RPMI with 2 mg·ml⁻¹ bovine serum albumin, and the upper wells contained 50 μ l cells at 1.5×10⁶ cells·ml⁻¹. The cells were preincubated for 30 min at 37°C with terbutaline or aminophylline, either at therapeutic levels (1, 2, 3 and 4 mg·l⁻¹, or 5, 10, 15 and 20 mg·l⁻¹, respectively), or in

separate experiments at higher concentrations (2, 4, 20, 40 and 160 mg·l⁻¹, or 10, 20, 50, 100, 200 and 400 mg·l⁻¹, respectively) before performing the assay. The upper and lower wells were separated by a 2 μ m pore polyvinylpyrrolidone (PVP)-free polycarbonate filter (Costar nucleopore, Costar UK, High Wycombe, UK). The chemotaxis chamber was incubated at 37°C for 20 min. The filter was then removed, and the upper surface wiped across a wiper blade (to remove any cells that had not migrated through the pores), followed by fixing and staining with Diff-quick (Baxter Incorp., UK). The cells which had migrated through the 2 μ m pores to the lower surface of the membrane were counted at ×400 magnification (five random fields per well, with three replicate wells). A mean value was obtained for each well and the average value for the replicate wells was taken as the result for that sample. In a further experiment to assess any additive effect of these agents on PMN chemotaxis the cells were preincubated both with terbutaline (4 mg·l⁻¹) and aminophylline (20 mg·l⁻¹) before performing the chemotaxis assay.

Superoxide assay

Superoxide release from PMNs in suspension was determined by measuring the superoxide dismutase inhibitable reduction of ferricytochrome C [10]. Again the PMNs were preincubated with therapeutic levels of terbutaline (1, 2, 3 and 4 mg·l⁻¹), or aminophylline (5, 10, 15 and 20 mg·l⁻¹), or at higher concentrations (2, 4, 20, 40 or 160 mg·l⁻¹ of terbutaline; 10, 20, 50, 100 or 400 mg·l⁻¹ of aminophylline), for 30 min at 37°C and then dispensed (10⁶ cells in 100 μ l) into the wells of a linbro plate (24-well, flat-bottomed, Flow laboratories). In order to assess any additive effect of these agents on superoxide generation, cells were also preincubated both with terbutaline and aminophylline (at 4 and 20 mg·l⁻¹, respectively) for 30 min before performing the assay. To reaction wells, either 10 μ l (5 mg·ml⁻¹) of superoxide dismutase (SOD, bovine erythrocytes, Sigma) was added to the cells to inhibit the reduction of cytochrome C or 10 μ l of reaction buffer (phosphate buffered saline 0.15 M, pH 7.2 containing 1 mM calcium chloride and 1 mM magnesium chloride). Horse heart ferricytochrome C (Sigma), in a volume of 100 μ l, was added to all wells to make a final concentration of 10 mM. All experiments were performed in the presence (stimulated cells) and absence (resting cells) of 1 μ M FMLP. The total volume of each well was made up to 1 ml with reaction buffer, with blank wells containing ferricytochrome C and reaction buffer alone. After 1 h incubation at 37°C in 5% CO₂/95% air, the supernatant from each well was recovered, centrifuged at 1,000×g to remove any cells, and the absorbance of each sample measured at 550 nm using a spectrophotometer. The amount of reduced cytochrome C was calculated using an extinction coefficient of 21.1 nM⁻¹ [11]. PMN superoxide generation was calculated as the difference in absorbance between reaction wells with and without SOD, and the results expressed as nmol superoxide released per hour per 10⁶ cells.

Fibronectin (FN) degradation

Degradation of fibronectin (FN) was assessed using the method of CAMPBELL *et al.* [12] modified by BURNETT *et al.* [1]. Purified human FN was obtained from the Sigma Chemical Co. (Poole, Dorset, UK) and iodinated by the chloramine-T method with sodium ^{125}I iodide (ICN Flow High Wycombe, Bucks, UK). The radiolabelled FN was diluted with unlabelled FN in 0.05 mol·l⁻¹ carbonate/bicarbonate buffer, pH 9.6, to give 2,000 cpm·μg⁻¹ FN, and dispensed into the wells of microtitre plates, at 30 μg·well⁻¹. The plates were allowed to dry at 37°C and then washed three times with phosphate buffered saline (pH 7.2) in order to remove any unbound iodine. The isolated PMNs suspended in RPMI medium (3×10^5 cells·well⁻¹) were preincubated with either terbutaline (4 mg·l⁻¹) or aminophylline (5, 10, 15 and 20 mg·l⁻¹) for 30 min at 37°C. Following the preincubation the cells were dispensed into the wells and the plates incubated at 37°C in a humidified atmosphere of 5% CO₂/95% air for 3 h. After incubation, the supernatant was collected from the wells and the proteolysed FN was measured by counting with an LKB Multigamma II gamma counter. The ^{125}I counts in the supernatants of wells containing RPMI medium alone (blank) were deducted from those containing PMNs. All experiments were performed in the absence (resting) and presence (stimulated cells) of 1 μM FMLP, and the result for each assay was determined from the mean value for three replicate wells. In the time course experiments of FN degradation by control cells and cells in the presence of 20 mg·l⁻¹ aminophylline, the supernatants were collected at various time-points (30, 60, 90, 120 and 180 min) from different wells and the amount of FN degraded calculated at each time-point.

Statistics

Statistical analyses of the effects of the agents on PMN function were assessed using analysis of variance.

Results

PMN chemotaxis

PMN chemotaxis to 10^{-8} M FMLP was increased slightly in a dose-dependent manner following preincubation of PMNs with terbutaline and aminophylline at therapeutic concentrations. The mean results of four experiments are summarized in figure 1. The results show an increase in chemotaxis ($p < 0.025$) from a mean control value of 22.5 (SEM 3.58) to 26.1 (4.73) cells·field⁻¹ with terbutaline 4 mg·l⁻¹, and to 26.3 (4.49) cells·field⁻¹ with aminophylline 20 mg·l⁻¹ ($p < 0.005$). Checkerboard analysis confirmed that the effect of these agents on PMN migration was due to an increase in chemotaxis, with no effect on chemokinesis (table 1). There was no evidence of any additive effect when cells were preincubated with both agents simultaneously (data not shown). In a further experiment, preincubation of the cells with higher

Fig. 1. — The effect of therapeutic concentrations of terbutaline and aminophylline on PMN chemotaxis to 10^{-8} M FMLP. Each histogram represents the mean of four experiments with SEM bars; $p < 0.005$ (ANOVA) with aminophylline and $p < 0.025$ with terbutaline compared to control. PMN: polymorphonuclear neutrophils; FMLP: formyl-methionyl-leucyl-phenylalanine; ANOVA: analysis of variance.

concentrations of terbutaline and aminophylline resulted in inhibition of PMN chemotaxis at terbutaline concentrations above 20 mg·l⁻¹, and aminophylline concentrations above 100 mg·l⁻¹, as summarized in table 2. Cell viability following 30 min preincubation with 160 mg·l⁻¹ of terbutaline and 400 mg·l⁻¹ aminophylline was still $>95\%$, as determined by the exclusion of trypan blue solution.

Superoxide anion generation

Preincubation of PMNs with terbutaline and aminophylline at therapeutic concentrations resulted in a dose-dependent increase in superoxide anion generation by

Table 1. — Checkerboard analysis of PMN chemotaxis to FMLP

Lower wells	Upper wells			
	Control medium	10^{-9} M	FMLP 10^{-8} M	10^{-7} M
a) Control cells				
Control medium	0	0	0	0
FMLP 10^{-9} M	10.2	0	0	0
FMLP 10^{-8} M	22.8	7.4	0	0
FMLP 10^{-7} M	5.4	14.5	14.3	0
b) Cells preincubated with aminophylline (20 mg·l⁻¹)				
Control medium	0	0	0	0
FMLP 10^{-9} M	10.6	0	0	0
FMLP 10^{-8} M	26.2	7.4	0	0
FMLP 10^{-7} M	5.8	17.8	14.4	0
c) Cells preincubated with terbutaline (4 mg·l⁻¹)				
Control medium	0	0	0	0
FMLP 10^{-9} M	11.8	0	0	0
FMLP 10^{-8} M	27.0	7.3	0	0
FMLP 10^{-7} M	4.0	15.6	13.3	0

Data are presented as mean cells·high power field⁻¹. FMLP: formyl-methionyl-leucyl-phenylalanine; PMN: polymorphonuclear neutrophils.

Table 2. — The effect of preincubation with aminophylline and terbutaline, at therapeutic and high concentrations, on FMLP-stimulated PMN chemotaxis (10^{-8} M FMLP) and superoxide anion generation (10^{-6} M FMLP)

Concentration	Cells-field ⁻¹	Superoxide generation nmol·10 ⁻⁶ cells·h ⁻¹
Aminophylline mg·l⁻¹		
Control	31.1±2.06	17.4
10 mg·l ⁻¹	36.9±0.27	20.15
20 mg·l ⁻¹	39.2±2.19	20.33
50 mg·l ⁻¹	40.1±0.77	17.54
100 mg·l ⁻¹	30.5±1.27	15.59
200 mg·l ⁻¹	18.8±0.20	-
400 mg·l ⁻¹	16.1±0.77	13.65
Terbutaline mg·l⁻¹		
Control	31.1±2.06	17.4
2 mg·l ⁻¹	30.8±1.04	17.44
4 mg·l ⁻¹	32.2±0.37	19.16
20 mg·l ⁻¹	20.6±0.40	14.79
40 mg·l ⁻¹	20.3±0.77	13.37
160 mg·l ⁻¹	18.3±0.82	8.11

*: Data are presented as the mean±SEM of three replicate wells. For abbreviations see legend to table 1.

resting cells and cells stimulated with 10^{-6} M FMLP. The effect of terbutaline (1–4 mg·l⁻¹) on PMN superoxide generation by resting and stimulated cells is shown in figure 2a and b, respectively. The results show an increase in superoxide production in a dose-dependent manner from a mean control value of 1.48 (SEM 0.44) to 1.76 (0.46) nmol·10⁻⁶ cells·h⁻¹ for unstimulated cells ($p<0.025$), and from a control value of 3.12 (1.06) to 3.77 (0.89) nmol·10⁻⁶ cells·h⁻¹ at a terbutaline concentration of 4 mg·l⁻¹ for stimulated cells ($p<0.005$).

The effects of aminophylline (5–20 mg·l⁻¹) on superoxide generation by resting PMNs and PMNs stimulated by FMLP are shown in figure 3a and b. Again there was a slight increase in superoxide production by PMNs in a dose-dependent manner. The results show an increase

from a mean control value of 2.12 (SEM 0.53) to 2.71 (0.58) nmol·10⁻⁶ cells·h⁻¹ at 20 mg·l⁻¹ for unstimulated cells ($p<0.0025$). When stimulated with 10^{-6} M FMLP the results increased from a control value of 4.43 (1.1) to 5.23 (0.95) nmol·10⁻⁶ cells·h⁻¹ ($p<0.005$) in the presence of 20 mg·l⁻¹ of aminophylline.

As with the chemotaxis results, there was no additive effect of these agents on PMN superoxide generation, when both agents were preincubated simultaneously with PMNs (data not shown).

In single experiments where the cells were preincubated with high concentrations of terbutaline and aminophylline, the results showed an inhibition of FMLP (10^{-6} M) stimulated superoxide anion generation (table 2). At a terbutaline level of 160 mg·l⁻¹, there was a reduction in superoxide release from a control value of 17.4 to 8.11 nmol·10⁻⁶ cells·h⁻¹. When the PMNs were preincubated with 400 mg·l⁻¹ aminophylline, there was again inhibition of superoxide release from a control value of 17.4 to 13.65 nmol·10⁻⁶ cells·h⁻¹.

FN degradation

Terbutaline had little effect on FN degradation when preincubated with PMNs. The mean result of four experiments had a control value for resting cells of 1.93 (SEM 0.38) µg and 1.79 (0.21) µg following preincubation of the PMNs with terbutaline (4 mg·l⁻¹; NS). When the cells were stimulated with 10^{-6} M FMLP the control value was 4.55 (1.2) µg and 4.34 (1.1) µg following preincubation of PMNs with terbutaline (4 mg·l⁻¹; NS).

Aminophylline preincubated with PMNs had no effect on FN degradation by resting or stimulated cells. Resting cells degraded a mean of 1.86 (SEM 0.47) µg of FN in the absence of aminophylline, with 1.86 (0.46) µg, 1.79 (0.45) µg, 1.82 (0.43) µg and 1.68 (0.32) µg in the presence of aminophylline at 5, 10, 15 and 20 mg·l⁻¹, respectively. When the cells were stimulated with 1 µM FMLP the results were 3.28 (0.65) µg of FN, 3.45 (0.74)

Fig. 2. — The increase of superoxide anion generation by PMNs preincubated with therapeutic concentrations of terbutaline. a) shows the effect on resting cells; and b) the effect when the cells were stimulated with 10^{-6} M FMLP. Each histogram represents the mean of five experiments with SEM bars; $p<0.025$ (ANOVA) for resting cells and $p<0.005$ for stimulated cells when compared to control. For abbreviations see legend to figure 1.

Fig. 3. — The potentiation of superoxide anion generation with therapeutic levels of aminophylline is shown for: a) unstimulated PMNs; and b) PMNs stimulated with 10^{-6} M FMLP. Each histogram represents the mean of five experiments with SEM bars; $p < 0.0025$ (ANOVA) for resting cells and $p < 0.005$ for stimulated cells compared to control. For abbreviations see legend to figure 1.

Fig. 4. — Time course of fibronectin degradation by resting polymorphonuclear neutrophils (PMNs), in the absence of aminophylline (—○—) and following preincubation of the PMNs with $20 \text{ mg} \cdot \text{l}^{-1}$ aminophylline (—●—). Each time-point represents the mean of five experiments with SEM bars.

μg , $3.67 (0.80) \mu\text{g}$, $3.59 (0.82) \mu\text{g}$ and $3.27 (0.76) \mu\text{g}$ with aminophylline at $0, 5, 10, 15$ and $20 \text{ mg} \cdot \text{l}^{-1}$, respectively. The results of time course experiments ($n=5$) of FN degradation by control cells and cells preincubated with $20 \text{ mg} \cdot \text{l}^{-1}$ aminophylline are shown in figure 4. At each of the sampled time-points, the amount of FN degraded in the presence of aminophylline was similar to the control values and at the 180 min time-point was $0.75 (\text{SEM } 0.08) \mu\text{g}$ for the control cells and $0.76 (0.10) \mu\text{g}$ for the aminophylline-treated cells (NS).

Discussion

The data presented support previous studies which have reported that methylxanthines have a biphasic effect on some PMN functions *in vitro* [6, 7]. In addition, our data show a similar biphasic effect with terbutaline on PMN chemotaxis and superoxide anion generation, but no effect on fibronectin degradation.

Therapeutic concentrations of both agents potentiated PMN chemotactic response to FMLP in a dose-dependent manner (15.0% with terbutaline at $4 \text{ mg} \cdot \text{l}^{-1}$ and 16.3% with aminophylline at $20 \text{ mg} \cdot \text{l}^{-1}$). On the other hand, preincubation with supratherapeutic doses of these agents resulted in a reduction of PMN chemotaxis by 41% with terbutaline ($160 \text{ mg} \cdot \text{l}^{-1}$) and 48% with aminophylline ($400 \text{ mg} \cdot \text{l}^{-1}$). Previous *in vitro* studies assessing the effects of theophylline and the β -agonist epinephrine on PMN migration report conflicting results, with some studies showing enhancement [13], whilst others reported no effect or inhibition of cell migration [5, 14]. We report stimulation of the PMN chemotactic response by aminophylline concentrations up to $50 \text{ mg} \cdot \text{l}^{-1}$ (from a control value of 31.1 ± 2.06 to 40.1 ± 0.77 cells-field⁻¹ at $50 \text{ mg} \cdot \text{l}^{-1}$), with inhibition at higher levels. This suggests a biphasic effect similar to that reported by other workers for superoxide production [6, 7], but confirms stimulation of the chemotactic response in the therapeutic range.

We have found similar effects of both terbutaline and aminophylline on PMN superoxide anion generation. At therapeutic concentrations of both agents, there was a small stimulatory effect in a dose-dependent manner for both resting cells (18.9% with terbutaline ($4 \text{ mg} \cdot \text{l}^{-1}$) and 27.8% with aminophylline ($20 \text{ mg} \cdot \text{l}^{-1}$) and cells that were stimulated with FMLP (20.8 and 18.1%, respectively). Our results also show inhibition of FMLP stimulated superoxide anion generation with the agents at concentrations above the therapeutic ranges (53.4% inhibition with terbutaline at $160 \text{ mg} \cdot \text{l}^{-1}$ ($0.58 \times 10^{-3} \text{ M}$), and 21.6% inhibition with $400 \text{ mg} \cdot \text{l}^{-1}$ aminophylline ($0.95 \times 10^{-3} \text{ M}$)), without an obvious effect on cell viability (as assessed by exclusion of trypan blue). These results support previous studies both with theophylline and aminophylline at therapeutic and supratherapeutic levels, in which the authors report stimulation of superoxide production, PMN aggregation and lysosomal enzyme release at 10^{-5} to 10^{-4} M , but up to 90% inhibition of these responses at concentrations $>10^{-4} \text{ M}$ [6, 7]. On the other hand, some workers have reported inhibition of oxygen metabolite

generation by up to 60% at therapeutic concentrations of theophylline administered to healthy volunteers over a 7 day period [14]. However, the inhibitory effect in this latter study was only seen when the respiratory burst was stimulated with calcium ionophore (A23187 or ionomycin), and not when stimulated with FMLP or the diacylglycerol oleoyl-acetyl-glycerol (OAG), suggesting the effect was related to a separate cell stimulatory mechanism.

In our *in vitro* studies, we have found no effect with either agent studied on PMN-mediated degradation of fibronectin. In contrast, some workers have reported a reduction in PMN degranulation following β -adrenergic stimulation of PMNs [4], which could be blocked by propranolol, whilst others report increased lysosomal release (30–50%) following preincubation of the cells with methylxanthines at therapeutic concentrations and inhibition at concentrations $>10^{-4}$ M [6]. The discrepancy of the above studies with our results may be due to methodological differences. Degranulation in the earlier studies [4, 6] was assessed following pretreatment of the PMNs with cytochalasin B, which itself can induce degranulation [4]. Our PMNs were not exposed to cytochalasin B and, thus, are more likely to reflect the possible effect *in vivo*.

The inhibitory effects of high concentrations of adrenergic agents and methylxanthines on PMN functions are well-established [4, 5], but their effects at therapeutic concentrations are more controversial. Both groups of agents raise intracellular cyclic adenosine monophosphate (cAMP) levels by stimulation of adenylyl cyclase or inhibition of phosphodiesterases, respectively. Elevation of cAMP levels are associated with inhibition of PMN functions, and it is possible that this accounts for the inhibitory effects at concentrations above the accepted therapeutic range. Adrenergic agents produce a concentration-dependent increase in adenylyl cyclase activity in the myocardium [15], but reduce adenylyl cyclase activity in platelets by 20–40% [16], whilst the action on PMNs is unclear. It seems likely from our results, that therapeutic concentrations of adrenergic agents may reduce adenylyl cyclase activity, with stimulation at higher levels leading to inhibition of cell responses.

Methylxanthines, as well as being inhibitors of cyclic nucleotide phosphodiesterase activity [17], are also competitive antagonists for adenosine A_1 and A_2 receptors [18]. Stimulation of the adenosine receptors selectively found on PMNs (A_2 receptors) results in inhibition of superoxide anion release following FMLP stimulation [19, 20]. Therefore, if stimulation of the A_2 receptor results in inhibition of PMN function, then competitive antagonism of the receptor would, potentially, have the opposite (stimulatory) effect. The accepted therapeutic levels of aminophylline and theophylline are comparable to the concentrations associated with adenosine receptor antagonism [21]. Thus, the results with therapeutic levels of aminophylline presented here may be due to A_2 receptor antagonism, whilst at higher concentrations the mechanism of inhibition of cell function is probably due to phosphodiesterase inhibition and elevation of cAMP. This theory is supported by a study comparing enprofylline (a methylxanthine with negligible adenosine antago-

nism) with theophylline [7]. Superoxide release was enhanced at low levels of theophylline ($1\text{--}100\text{ }\mu\text{mol}\cdot\text{l}^{-1}$) but inhibited at high concentrations ($300\text{ }\mu\text{mol}\cdot\text{l}^{-1}$), whereas enprofylline led only to a concentration-dependent inhibition of superoxide production. Thus, the controversy in the literature regarding possible inhibitory or stimulatory effects of these agents probably relates to differences in concentrations used in the various studies.

In summary, the results presented here show a biphasic effect of β_2 -agonists and methylxanthines on PMN function *in vitro*. Both agents appear to stimulate neutrophil chemotactic response and superoxide generation at therapeutic concentrations. Since the recruitment of PMNs to the lung and superoxide generation have been implicated in the development of lung damage, these agents may be detrimental in situations where no clear clinical benefit has been achieved. On the other hand, the use of these agents during acute lung infections may even prove beneficial, by facilitating PMN recruitment to the lung and increasing bacterial killing during the episode. Further studies of the effect of these agents on PMN function *in vivo* are required in order to fully assess their role in chronic lung disease, and determine whether the relatively small effects reported here are clinically important in patients with chronic bronchitis and emphysema.

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