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Anti-inflammatory effects of theophylline, cromolyn and salbutamol in a murine model of pleurisy

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1 The aim of this study was to examine the effect of theophylline, cromolyn and salbutamol, three well-known anti-asthmatic drugs, on the early (4 h) and late (48 h) phases of cell migration and fluid leakage induced by carrageenin in the pleural cavity of mice.

2 In the first set of experiments, animals were pretreated (30 min) with different doses of theophylline (0.5–50 mg kg^{-1}, i.p.), cromolyn (0.02–0.2 mg per pleural cavity) or salbutamol (0.05–50 mg kg^{-1}, i.p.); the total and differential cell content, and also the exudate were analysed 4 h after carrageenin (1%) administration. Afterwards, in order to evaluate the time course effects of these drugs on both phases of the inflammatory reaction, one dose employed in the above protocol was chosen, to pretreat (0.5–24 h) different groups of animals. The studied parameters were evaluated 4 and 48 h after pleurisy induction.

3 Acute administration of theophylline (1–50 mg kg^{-1}, i.p.), cromolyn (0.02–0.2 mg per pleural cavity) and salbutamol (0.5–50 mg kg^{-1}, i.p.), 30 min prior to carrageenin, caused significant inhibition of total cell and fluid leakage in the pleural cavity at 4 h ($P<0.01$). All drugs exerted a long-lasting inhibitory effect on both exudation and cell migration ($P<0.01$) when administered 0.5–8 h before pleurisy induction. However, the temporal profile of the inhibitory effect induced by these drugs on the first phase of the inflammatory reaction was clearly different. Thus, the inhibitory effect induced by theophylline and cromolyn on exudation was significantly longer (up to 24 h) in comparison to their effects on cell migration (only up to 8 h). In contrast, although salbutamol when administered 30 min before pleurisy induction abolished fluid leakage ($P<0.01$), this effect was not sustained in the groups pretreated for 4–8 h. In these latter groups, a significant but much smaller reduction of exudation was observed ($P<0.01$), whereas the magnitude of cell migration inhibition did not vary.

4 The second phase (48 h) of the inflammatory reaction induced by carrageenin (1%) was significantly inhibited by cromolyn (0.02 mg per pleural cavity) when this drug was administered 0.5–24 h before pleurisy induction ($P<0.01$). Similar results were observed when theophylline (50 mg kg^{-1}, i.p.) was administered 0.5–4 h before the injection of the phlogistic agent ($P<0.01$). Treatment of the animals with salbutamol (5 mg kg^{-1}, i.p.), 0.5–24 h before pleurisy induction, did not inhibit either cell migration or fluid leakage. In this condition, a significant increase of these parameters was observed in the group pretreated with salbutamol 8–24 h before pleurisy induction ($P<0.01$).

5 These results indicate that theophylline and cromolyn were able to inhibit the early (4 h) and late (48 h) phases of the inflammatory reaction induced by carrageenin in a murine model of pleurisy. Salbutamol was effective only against the early phase. The inhibitory effects of theophylline, cromolyn and salbutamol on the early phase of this inflammatory reaction were long-lasting, although a distinct profile of inhibition was observed among them. These findings confirm and extend previous results described in other models of asthma and support both clinical and experimental evidence suggesting that these anti-asthmatic agents exhibit marked anti-inflammatory properties.

Keywords: Mouse pleurisy; carrageenin; inflammation; theophylline; cromolyn; salbutamol; anti-asthmatic drugs

Introduction

Data reported in the literature have provided a great amount of evidence that airway inflammation is the primary event in asthma and that airway hyperresponsiveness, both transient and persistant, is a secondary event to the airway inflammation (O'Byrne *et al.*, 1987; Beasley *et al.*, 1989; Smith, 1992; Aalbers *et al.*, 1993). In addition, the increase in the understanding of the importance of the inflammatory response in the airways was associated with a review of the therapeutic approach to asthmatic patients. So, in parallel with a dramatic change in the treatment of these patients (Barnes, 1989; Kemp, 1993), the interest in understanding further the mechanisms by which the conventional therapy, namely theophylline, cromolyn, and β_2-adrenoceptor agonists, influence this inflammatory response has also been increased (Erjefalt & Persson, 1991).

Nowadays, there is considerable evidence to support an anti-inflammatory role for theophylline in asthma (Milgrom & Bender, 1993; Sullivan *et al.*, 1994). It has been demonstrated that this drug is able to suppress, both *in vitro* and *in vivo*, a variety of inflammatory and immune cell functions (Torphy & Undem, 1991). For instance, it has been shown that theophylline reduces the phagocytic and bactericidal capacities of polymorphonuclear leukocytes and macrophages, the release of reactive oxygen species by macrophages, the proliferative response of T lymphocytes to mitogens and the cytotoxic activity of natural killer as well as tumour necrosis factors release from activated human mononuclear cells (Spatafora *et al.*, 1994). Moreover, the lung anti-inflammatory properties of cromolyn have also been reviewed (for review see: Barnes, 1989; Bernstein & Bernstein, 1993). Thus, besides its potent anti-allergic effect which inhibits the release of anaphylactic mediators from mast cells (Tainsh *et al.*, 1991; Okayama & Church, 1992), another proposed mechanism of action of cromolyn is its ability to inhibit neural reflex bronchoconstriction induced by several neuropeptides that take part in the

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neurogenic inflammatory process (Morley, 1994). This would result in a marked inhibition of the inflammatory cells and consequently of the release of several mediators which, in turn, would attenuate their spasmogenic effects on smooth muscle tone (Bernstein & Bernstein, 1993). In relation to β_2-adrenoceptor agonists like salbutamol, the fact that this drug exerts a potent inhibitory effect on the first phase of airway hyperresponsiveness in experimental and clinical studies indicates that it also acts by inhibiting some steps of the inflammatory response (Pauwels & Person, 1991; Pacheco *et al.*, 1992). In general, attempts to characterize in more detail the effects of these drugs in experimental animal models of asthma have provided conflicting results, probably due to the fact that this disease has a multifactorial aetiology (Becker & Bierman, 1994). This observation thus limits the development of a model that best represents clinical asthma.

Recent evidence suggests that rat pleurisy is a useful model for characterizing and/or screening new anti-inflammatory drugs (Brito, 1989), since it is possible to evaluate simultaneously both total and differential cell count as well as the amount of exudation in a closed cavity (Lo *et al.*, 1982). Although the mouse pleurisy model induced by carrageenin does not fit the criteria of an asthma model, the inflammatory response induced by this phlogistic agent in the pleural cavity shows some specific characteristics that are similar to those described for this disease. It is well-known that allergen bronchoprovocation in patients with asthma produces a biphasic response consisting of immediate bronchospasm which almost resolves within minutes and can be prevented by treatment with β_2-adrenoceptor agonists. Some minutes after exposure to the allergen, a late episode of bronchoconstriction can also occur, which is more severe than the immediate response and which is resistant to treatment with β_2-adrenoceptor agonists (Hargreaves *et al.*, 1981; Paggiaro *et al.*, 1991). These early and late responses are associated with a marked inflammatory reaction in the airways as a result of the release of distinct mediators and activation of different types of immune cells (O'Byrne *et al.*, 1987; Schlosberg *et al.*, 1993). In this context, the mouse pleurisy model induced by carrageenin is typically associated with an early and a late inflammatory reaction in response to the phogistic challenge, where several mediators such as histamine, bradykinin, prostaglandins, PAF and leukotrienes are involved (Henriques, 1993). Thus, the aim of this study was to analyse and compare the effect of theophylline, cromolyn and a β_2-adrenoceptor agonist, salbutamol, in a murine model of pleurisy.

Methods

Animals

Non-fasted 2-month old adult Swiss mice of both sexes (18–25 g) were used. The animals were maintained in an environment of controlled temperature (21 ± 2°C), illuminated by daylight supplemented by electric light from 06 h 00 min to 18 h 00 min, with free access to food and water.

Induction of pleurisy

Twenty-four hours prior to the experiments, animals were challenged with a solution of Evans blue dye (25.0 mg kg^{-1}, 0.2 ml, i.v.) in order to evaluate further the degree of exudation in the pleural space (Henriques *et al.*, 1992). On the following day, animals were lightly anaesthetized with ether, and carrageenin (1%) was injected into the right pleural space. The animals were killed at different periods of time (1–100 h) with an overdose of ether, and immediately after opening the thorax, the pleural cavity was washed with 1 ml of phosphate buffered saline (PBS, pH 7.6, composition: NaCl 137 mM, KCl 2 mM and phosphate buffer 10 mM) plus heparin (20 iu ml^{-1}) and the volume collected with automatic pipettes. Total leukocyte counts were performed in Neubauer chambers by means of an optical microscope after diluting the pleural fluid with Türk solution (1 : 200). Cellular smears were stained with May-Greenwald-Giemsa for differential analysis which was performed under immersion objective. A sample of the collected fluid (500 μl) from the pleural space was separated and

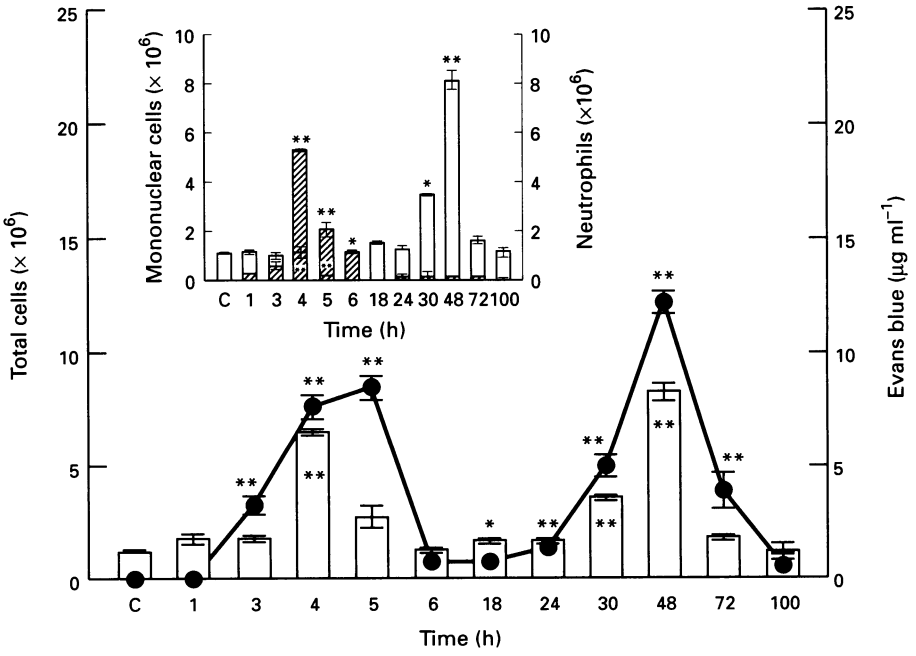

Figure 1 Time course profile of total cell content and Evans blue extravasation induced by carrageenin in the pleural cavity of mice. Control responses (C) in animals injected only with sterile saline. Asterisks inside and outside the columns indicate the statistical significance of both total cell content (columns) and exudate values (●) in comparison to C. The inset shows the variation of both mononuclear (open columns) and neutrophil cells (hatched columns) along the time scale in the mouse pleurisy model induced by carrageenin. Asterisks inside and outside the columns indicate the statistically significant differences of both neutrophil and mononuclear cells in comparison to C. Each column and (●) represents the mean of 6 to 10 animals with s.e.means also shown. In some groups, the s.e.mean values are smaller than the symbol. $*P<0.05$ and $**P<0.01$.

stored in the freezer (−20°C) to determine the concentration of Evans blue dye. On the day of the experiments, a batch of samples was defrosted to room temperature, and the amount of dye was then estimated by colorimetry (Compu-Espectro Spectrometer, Brazil) at 600 nm by interpolation from a standard curve constructed to Evans blue in the range of 0.01–50 $\mu g\ ml^{-1}$.

Experimental procedures

Considering that the inflammatory response induced by carrageenin (1%) in the pleural space of the mice has a biphasic profile, peaking at 4 and 48 h after pleurisy induction, these time points were chosen to analyse the effect of the drugs studied. In preliminary experiments (results not shown), sev-

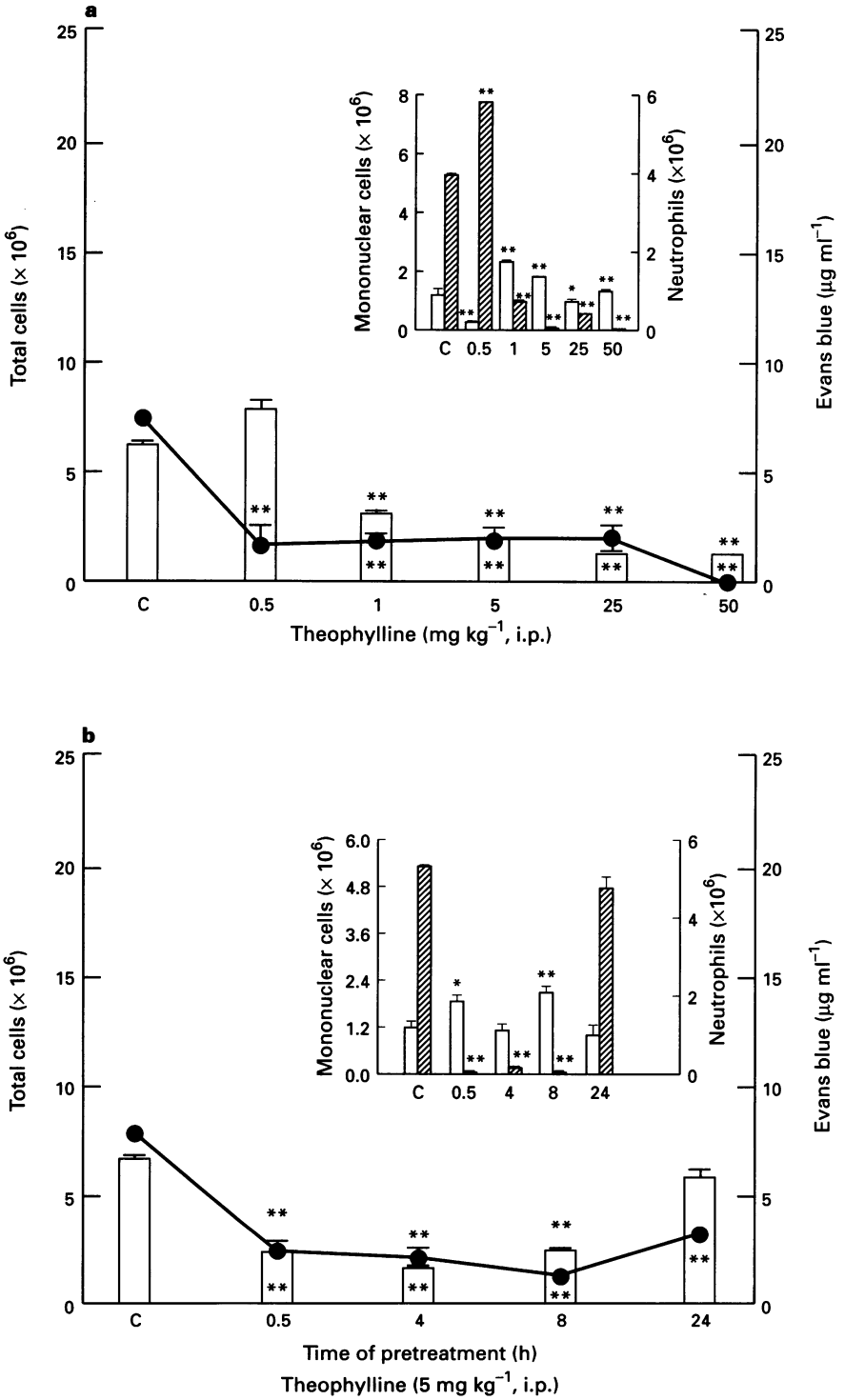

Figure 2 Effect of theophylline on the first phase (4 h) of mouse pleurisy induced by carrageenin. (a) Effect of different doses (0.5–50.0 mg kg^{-1}, i.p.) administered 30 min prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks outside and inside the columns indicate the statistically significant differences of both total cell content (columns) and exudate levels (●) in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil (hatched columns). (b) Effect of theophylline (5 mg kg^{-1}, i.p.) administered 0.5–24 h prior to pleurisy induction under the same experimental conditions. Asterisks outside and inside the columns indicate the statistically significant differences of both total cell content and exudate levels in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). Each column and (●) represents the mean of 6 to 10 animals, with s.e.means also shown. In some groups, s.e.mean values are smaller than symbol. $*P<0.05$ and $**P<0.01$.

eral doses of each drug and different intervals of pretreatment were tested to determine the best period of pretreatment in the analysis of their effects in this model. Based on this protocol, the period of 30 min prior to carrageenin injection was chosen for use in the following experiments. Thus, the animals were treated with theophylline (0.5–50 mg kg^{-1}, intraperitoneally-i.p.), cromolyn (0.02–0.2 mg per pleural cavity) or salbutamol (0.05–50 mg kg^{-1}, i.p.), 30 min before pleurisy induction, and indices of inflammation were analysed after 4 h. In other experiments, an estimate of the duration of the inhibitory effect caused by the putative anti-inflammatory agents was evaluated indirectly by pretreating the animals 0.5–24 h before pleurisy with a single dose of each of the drugs studied in the experiments above; animals were killed at 4 h. The possible effect of these drugs on the late phase of cellular migration and fluid leakage to the pleural cavity (48 h) was also evaluated by pretreating the animals with one of the doses of theophylline, cromolyn or salbutamol used in the previous experiments. In this condition, an estimate of the temporal profile of these drugs was also evaluated by pretreating the animals 0.5–24 h before pleurisy induction.

Statistical analysis

Data are reported as mean ± s.e.mean. Differences between groups were determined by analysis of variance (ANOVA) complemented with Dunnett's or Newmann-Kewl's tests when indicated. P less than 0.05 was considered significant.

Drugs

The following drugs were used: lambda carrageenin (degree IV), cromolyn (disodium cromoglycate), salbutamol, theophylline (Sigma Chemical Co, St. Louis, MO, U.S.A.), heparin (Liquemine, Roche, Brazil) and Evans blue (Merck, Brazil). All salts used were Merck high purity grade reagents and the sterile saline solution (NaCl 0.9%) was obtained from different commercial sources. On the day of the experiments, the drugs were diluted in sterile saline at room temperature, except for theophylline solution which was heated at 37°C for 5 min.

Results

Pleurisy induced by carrageenin in the mouse pleural cavity

As ascertained before (Henriques, 1993), carrageenin induced a significant biphasic increase in both leukocyte number and exudation in the pleural cavity. These two phases of the inflammatory response to carrageenin in the mouse pleural cavity (Figure 1) were named 'first' (or 'early') and 'second' (or 'late'). Thus, following carrageenin administration, a gradual enhancement in the total number of cells was observed, due primarily to neutrophils, that peaked at 4 h ($P<0.01$). A sudden decrease of these cells was observed 5 h before pleurisy induction, the total cell content being close to, or within, the normal range up to 24 h. A parallel increase in fluid leakage to the pleural cavity was also observed, which peaked between 4 and 5 h after pleurisy induction ($P<0.01$). The exudate volume remained minimal when analysed between 6–24 h after triggering the inflammatory process.

A second peak in both cell infiltration and fluid leakage was observed between 30 and 72 h ($P<0.01$), peaking at 48 h. At this time, the total number of cells did not differ from those observed at 4 h, although the differential cell count had revealed an enhancement due mainly to mononuclear cells ($P<0.01$) (Figure 1). In contrast, the amount of fluid leakage 48 h after pleurisy induction was higher than that observed at 4 h ($P<0.01$). A complete resolution of the inflammatory process was oberved 100 h after pleurisy induction.

Effect of theophylline, cromolyn and salbutamol on carrageenin-induced pleurisy in mice

Figure 2a shows the effect of theophylline (0.5–50 mg kg^{-1}, i.p.) on the first phase of the inflammatory reaction (4 h). Doses between 1 and 50 mg kg^{-1}, i.p., significantly inhibited the total cell migration, which was primarily composed of neutrophils ($P<0.01$). This effect was not dose-dependent. The dose of 0.5 mg kg^{-1}, i.p., was without effect on the response of cell migration. In addition, all tested doses of theophylline caused a significant reduction of exudation ($P<0.01$). Pretreatment of the animals (0.5–24 h) with theophylline

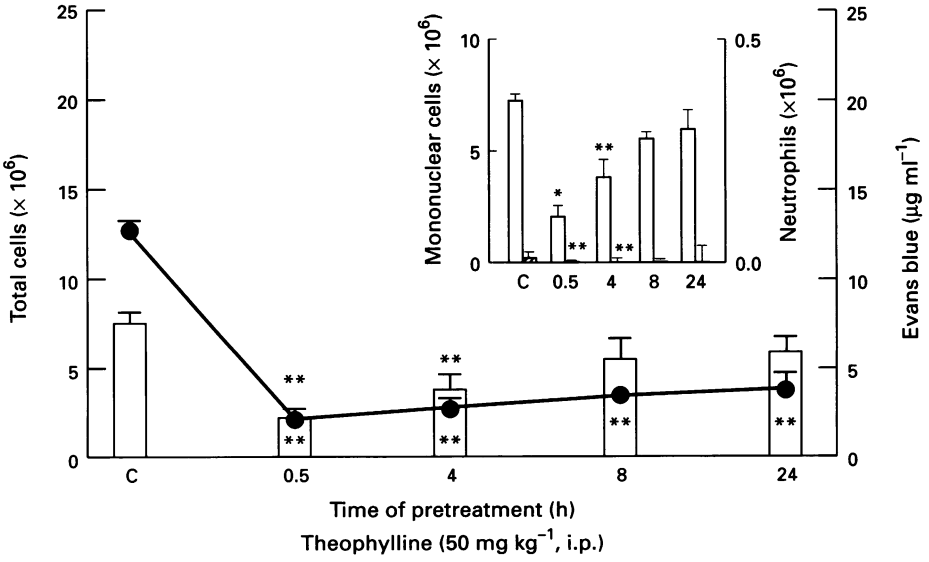

Figure 3 Effect of theophylline (50 mg kg^{-1}, i.p.) on the second phase (48 h) of the inflammatory reaction induced by carrageenin in the mouse pleural cavity administered 0.5–24 h prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks inside and outside the columns indicate the statistically significant differences of both exudate values (●) and total cell content (columns) in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). Each column and (●) represents the mean of 6 to 10 animals with s.e.mean also shown. In some groups, the s.e.mean values are smaller than the symbol. $^{**}P<0.01$.

(5 mg kg^{-1}, i.p.) revealed that this drug caused a long-lasting (8 h) inhibitory effect on both fluid leakage and cell migration that occurred in the first phase of this inflammatory reaction (Figure 2b). However, the inhibitory effect induced by this drug on fluid leakage lasted longer (24 h) in comparison to cell migration which was significantly inhibited only when the animals were treated up to 8 h before pleurisy induction. This dose of theophylline (5 mg kg^{-1}, i.p.) failed to affect the late cell influx (48 h), although an inhibition of exudation was still observed when animals were pretreated 0.5–24 h before

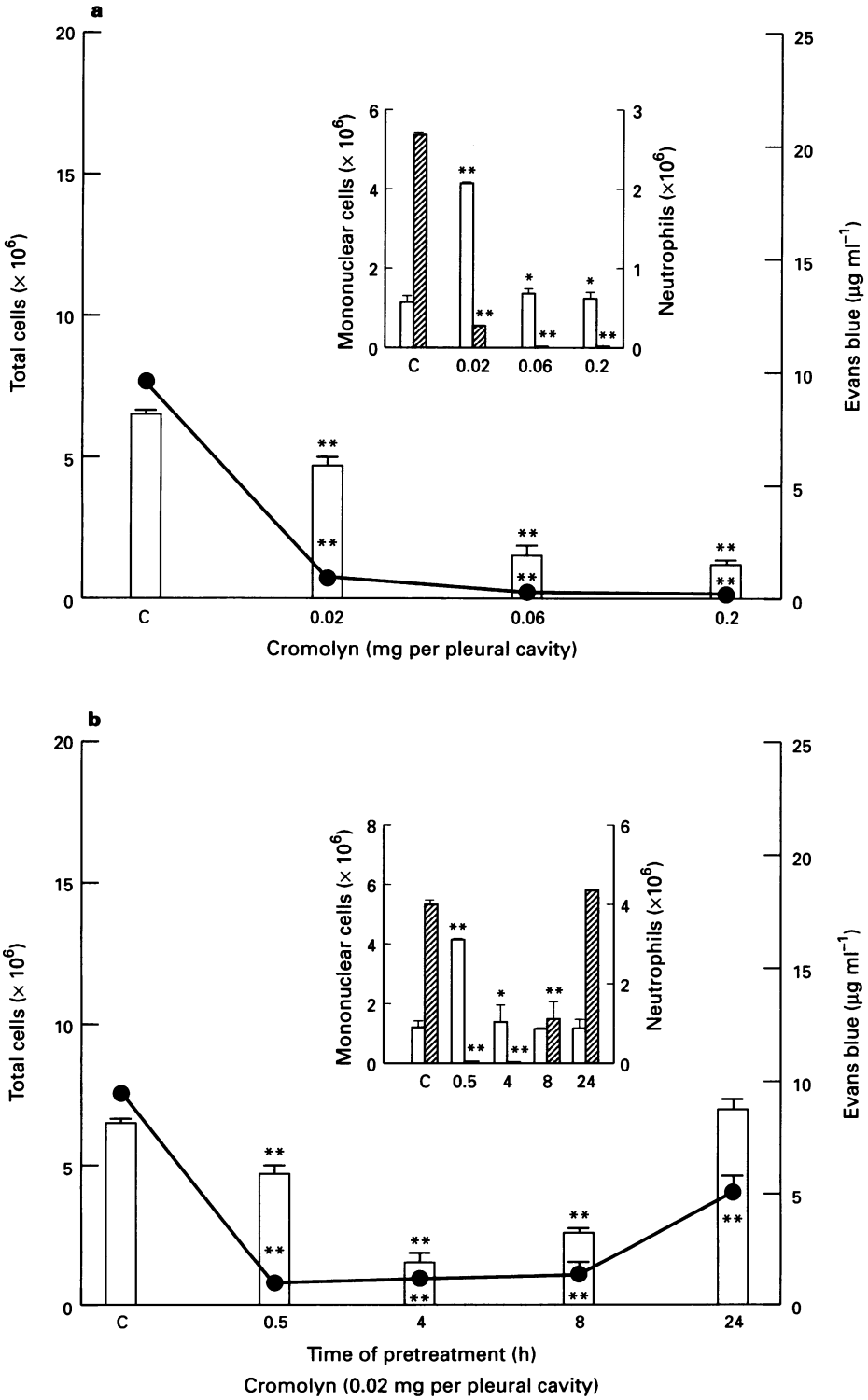

Figure 4 Effect of cromolyn on the first phase (4 h) of mouse pleurisy induced by carrageenin. (a) Effect of different doses (0.02–0.2 mg per pleural cavity) administered 30 min prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks inside and outside the columns indicate the statistically significant differences of both exudate values (●) and total cell content (columns) in comparison to C. Inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). (b) Effect of cromolyn (0.02 mg per pleural cavity) administered 0.5–24 h prior to pleurisy induction under the same experimental conditions. Asterisks inside and outside the columns indicate the statistically significant differences of both exudate values (●) and total cell content (columns) in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil cells. (hatched columns). Each column and (●) represents the mean of 6 to 10 animals with s.e.mean also shown. In some groups, the s.e.mean values are smaller than the symbol. $*P<0.05$ and $**P<0.01$.

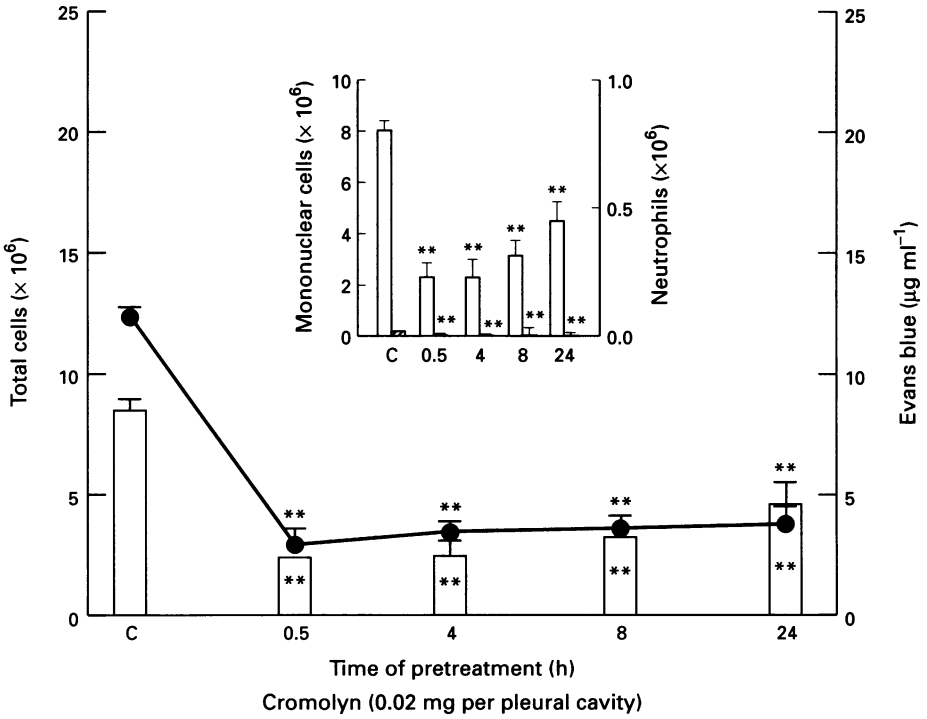

Figure 5 Effect of cromolyn (0.02 mg per pleural cavity) on the second phase (48 h) of the inflammatory reaction induced by carrageenin in the mouse pleural cavity when administered 0.5–24 h prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks inside and outside the columns indicate the statistically significant differences of both exudate values (●) and total cell content (columns) in comparison to C. The inset shows the drug effects on mononuclear (open columns) and neturophil cells (hatched columns). Each column and (●) represents the mean of 6 to 10 animals with s.e.mean also shown. In some groups, the s.e.mean values are smaller than the symbol. $^{**}P<0.01$.

($P<0.01$) (results not shown). However, a higher dose of theophylline (50 mg kg^{-1}, i.p.), administered either 0.5 or 4 h before pleurisy induction, significantly inhibited the late phase (48 h) response. Again, theophylline exhibited a long-lasting inhibitory effect on exudation, which remained significantly inhibited for up to 24 h after pretreatment (Figure 3).

Figure 4a shows that all tested doses of cromolyn (0.02–0.2 mg per pleural cavity, 30 min) were effective in inhibiting the first phase of the inflammatory process ($P<0.01$) (Figure 4b). The intrapleural administration of cromolyn (0.02 mg per pleural cavity) up to 8 h before pleurisy induction resulted in inhibition of both fluid leakage and cell migration ($P<0.01$). The inhibitory effect induced by cromolyn on exudation was significantly longer lasting (up to 24 h) in comparison to its effects on cell migration (only up to 8 h). On the other hand, the same dose of cromolyn (0.02 mg per pleural cavity) was also effective in inhibiting the late inflammatory response (48 h) induced by carrageenin in the pleural cavity (Figure 5).

The effect of salbutamol (0.05–50 mg kg^{-1}, i.p., 30 min before) on the first phase of the mouse pleurisy induced by carrageenin is shown in Figures 6a and b. Both cell migration and exudation were significantly inhibited ($P<0.01$), although this was not dose-dependent. Salbutamol (5 mg kg^{-1}, i.p.) given 0.5 to 8 h, (but not 24 h) before carrageenin, caused a significant reduction of both cell migration and exudation ($P<0.01$) (Figure 6b). In this situation, fluid leakage was abolished only in the group treated with β_2-agonist 30 min prior to carrageenin ($P<0.01$). As shown, this inhibitory effect was not sustained, since pretreatment of the animals (4–8 h) with the same dose of salbutamol resulted in a much smaller reduction of fluid leakage. Under the same experimental conditions, treatment of the animals with salbutamol (5 mg kg^{-1}, i.p.) 24 h before pleurisy induction did not affect cell migration or fluid leakage (Figure 6b). In relation to the second phase of the inflammatory reaction, salbutamol (5 mg kg^{-1}, i.p.), administered 0.5 to 24 h prior to carrageenin, did not inhibit either cell migration or fluid leakage (Figure 7). Moreover, 48 h after pleurisy induction, a slight but significant increase of both cell migration and exudation was observed in the animals pretreated 8–24 h with salbutamol ($P<0.01$) (Figure 7).

Discussion

The results of the present study clearly show that theophylline and cromolyn elicited a long-lasting, anti-inflammatory effect, being able to inhibit both the early and the late phases of cell influx as well as fluid exudation in a murine model of pleurisy. In addition, salbutamol, a β_2-adrenoceptor agonist, was also able to inhibit the first phase of the inflammatory reaction induced by carrageenin in the pleural cavity, but it was ineffective in suppressing the late response. Taken together, these data further support previous evidence that these drugs possess anti-inflammatory properties (Cresciolli *et al.*, 1991; Gern & Lemanske, 1993; Becker & Bierman, 1994).

In this study, acute treatment of mice with theophylline was able to inhibit significantly both the first and second phases of the inflammatory reaction induced by carrageenin. These data also provides evidence that this drug exerts a long-lasting effect since when it was administered either 0.5 or 4 h before carrageenin, it markedly inhibited both exudation and cell migration which peaked 4 and 48 h after pleurisy induction. However, the dose of theophylline which inhibited the late inflammatory reaction was ten fold higher (50 mg kg^{-1}, i.p.) than that necessary to inhibit the first phase, suggesting the possible involvement of different inflammatory mediators in this model, or even differences in the metabolism of this drug (Nicholas *et al.*, 1993). These findings seem to be relevant because few studies have addressed the putative anti-inflammatory properties of theophylline in experimental models unrelated to asthma (Yukawa *et al.*, 1989; Schrier *et al.*, 1990; Svenjo, 1990). Considering that the inflammatory reaction induced by carrageenin involves the participation of several mediators and cell types (Brito, 1989), it is unlikely that this

drug is acting in a selective fashion, but rather in a common step of this process that is shared by several mediators and/or cells. Thus, it is possible that the well-known putative mechanisms of action proposed for this drug, such as inhibition of 3′-5′-cyclic adenosine monophosphate-phosphodiesterase (PDE) or cyclic guanosine monophosphate-PDE, (Milgrom, 1993), antagonism of purinoceptors and/or blockade of calcium influx, are not the sole pathways involved in this in-

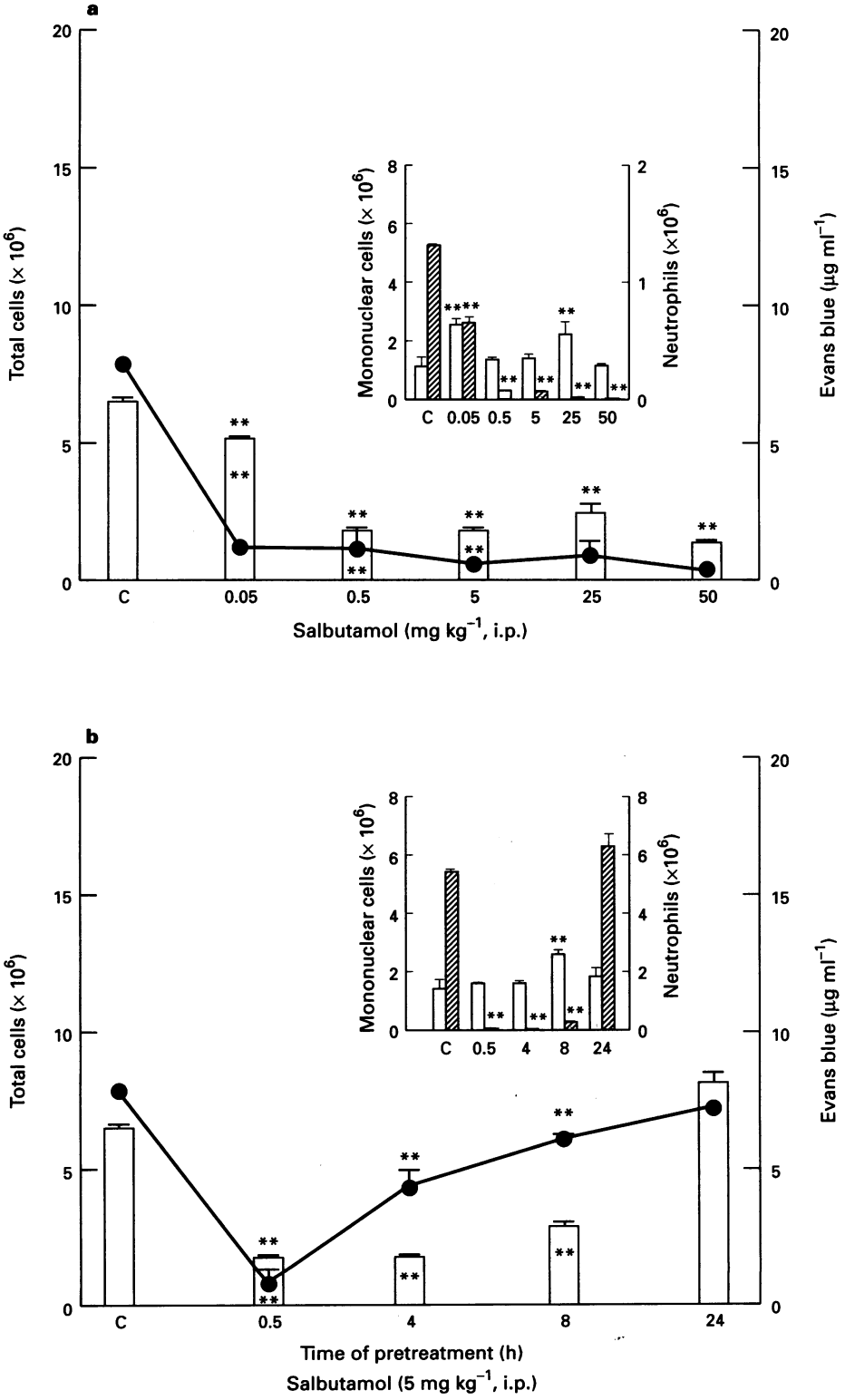

Figure 6 Effect of salbutamol on the first phase (4 h) of mouse pleurisy induced by carrageenin. (a) Effect of different doses (0.05–50 mg kg^{-1}, i.p.) administered 30 min prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks inside and outside the columns indicate the statistically significant differences of both exudate values (●) and total cell content (columns) in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). (b) Effect of salbutamol (5 mg kg^{-1}, i.p.) administered 0.5–24 h prior to pleurisy induction under the same experimental condition. Asterisks inside and outside the columns indicate the statistically significant differences of both total cell content (columns) and exudate values (●) in comparison to C. The inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). Each column and (●) represents the mean of 6 to 10 animals with s.e.mean also shown. In some groups, the s.e.mean values are smaller than the symbol. $^{**}P<0.01$.

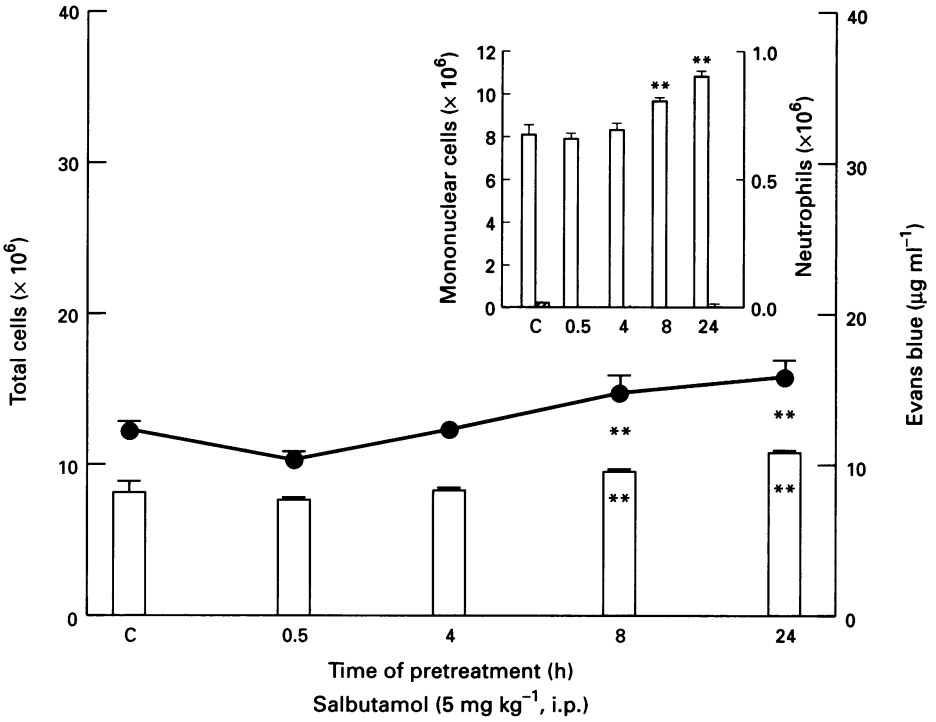

Figure 7 Effect of salbutamol (5 mg kg^{-1}, i.p.) on the second phase (48 h) of the inflammatory reaction induced by carrageenin in the mouse pleural cavity when administered 0.5–24 h prior to pleurisy induction. Control responses (C) in animals injected with carrageenin. Asterisks inside and outside the columns indicate the statistical differences of both total cell content (columns) and exudate levels (●) in comparison to C. Inset shows the drug effects on both mononuclear (open columns) and neutrophil cells (hatched columns). Each column or (●) represents the mean of 6 to 10 animals, with s.e.mean also shown. In some groups the s.e.mean values are smaller than the symbol. $^{**}P<0.01$.

hibitory effect. Torphy & Undem (1991) pointed out that of theophylline's many activities, phosphodiesterase inhibition may be the most important, and that this class of compounds should possess anti-inflammatory activity. This is suggested both in clinical (Sullivan *et al.*, 1994) and experimental (Raeburn *et al.*, 1994; Banner & Page, 1995) studies, where it was demonstrated that chronic treatment with both theophylline and PDE inhibitors was able to attenuate the airway inflammatory response to allergen inhalation. However, although our data support the potential role of theophylline as an anti-inflammatory drug, further studies will be necessary to achieve a clear understanding of the mechanisms underlying its action.

Cromolyn, when administered intrapleurally, also exhibited marked anti-inflammatory effects in this model. The drug inhibited the cell migration and leakage associated with both the early and late phase reactions. These findings suggest that the anti-inflammatory properties of cromolyn are complex and its mechanism of action may involve other targets in addition to mast cells (Bernstein & Bernstein, 1993). The results of our study are in accordance with other data from the literature, where it was demonstrated that this drug markedly inhibited both microvascular leakage and cell migration in several models of bronchial hyperreactivity (Pauwels, 1989; Tarayre *et al.*, 1991). Taken together, the results presented here do not permit us to suggest the precise mechanism by which cromolyn is exerting its anti-inflammatory effects, but one cannot discard the possibility that this drug may be acting in one or several steps of neurogenic inflammation as has been recently proposed (Kowaski *et al.*, 1990; Holian *et al.*, 1991).

Salbutamol caused a significant inhibition of both exudation and cell migration of the first phase of the inflammatory reaction when the animals were pretreated 0.5–8 h before the administration of carrageenin. In this situation, whereas cell content remained markedly reduced, the volume of harvested fluid in the pleural cavity was much less inhibited in comparison to that obtained when the animals were treated 30 min prior to carrageenin. Furthermore, in contrast with theophylline and cromolyn, salbutamol was not effective in inhibiting the late phase of the inflammatory response induced by carrageenin in the mouse pleurisy model. These findings are in agreement with those described in the literature where it was demonstrated that β_2-adrenoceptor agonists inhibit only the first phase of the inflammatory reaction either in experimental or in human assays of bronchoprovocation with different allergens or mediators (Pacheco *et al.*, 1992; Yamada *et al.*, 1992; Church *et al.*, 1993). Based on this evidence, we cannot discard the possibility that the inhibitory effect induced by salbutamol on the first phase of this inflammatory reaction might be related to its action on mast cells (Page, 1991). In addition, the failure of this drug to inhibit the second phase of the inflammatory reaction induced by carrageenin could be the result of its short half-life in comparison to the other drugs (Gern & Lemansk, 1993). Further studies to clarify this possibility will be necessary.

In summary, our results confirm in another model of inflammation that theophylline and cromolyn exhibit a marked and long-lasting anti-inflammatory profile, both drugs being able to inhibit both exudation and cell migration in a murine model of pleurisy. The β_2-adrenoceptor agonist, salbutamol, also inhibited the early inflammatory response, although it was ineffective in modifying the late response. The anti-inflammatory profile presented by the three drugs in the present study is in accordance with those described in other models, especially those related to asthma. Finally, the induction of pleurisy in the mouse provides an alternative and interesting option in the study of the mechanism of anti-asthmatic drugs.

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References

AALBERS, R., SMITH, M. & TIMENS, W. (1993). Immunohistology in bronchial asthma. *Respir. Med.*, **87B:** (suppl.) 13–21.

BANNER, K.H. & PAGE, C. (1995). Acute versus chronic administration of phosphodiesterase inhibitors on allergen-induced pulmonary cell influx in sensitized guinea-pigs. *Br. J. Pharmacol.*, **114,** 93–98.

BARNES, P.J. (1989). A new approach to the treatment of asthma. *N. Engl. J. Med.*, **321,** 1517–1527.

BEASLEY, R., ROCHE, W.R., ROBERTS, J.A. & HOLGATE, S.T. (1989). Cellular events in the bronchi in mild asthma and after provocation. *Am. Rev. Respir. Dis.*, **139,** 806–817.

BECKER, J.W. & BIERMAN, C.W. (1994). Prophylactic anti-asthma drugs. In *Drugs in the Lung*. ed. Page, C.P. & W.J. Metzer, C.P., pp. 221–249, New York: Raven Press.

BERNSTEIN, J.A. & BERNSTEIN, I.L. (1993). Cromolyn and nedocromil. *Immunol. Allergy Clin. North Am.*, **13,** 891–902.

BRITO, F.B. (1989). Pleurisy and pouch models of acute inflammation. In *Pharmacological Methods in the Control of Inflammation*. ed. Liss, A. R., pp. 173–228, Dagenham: Rhone Poulenc Ltd.

CRESCIOLLI, S., SPINAZZI, A., PLEBANI, M., MAPP, C.E., BOSCHETTO, P. & FABBRI, L.M. (1981). Theophylline inhibits early and late asthmatic reactions induced by allergens in asthmatic subjects. *Ann. Allergy*, **66,** 245–251.

CHURCH, M.K., HUTSON, P.A. & HOLGATE, S.T. (1993). Nedocromil sodium blocks the early and late phases of allergen challenge in a guinea pig model of asthma. *J. Allergy Clin. Immunol.*, **92,** 177–182.

ERJEFALT, L. & PERSSON, C.G. (1991). Pharmacological control of plasma exudation into tracheobronchial airways. *Am. Rev. Respir. Dis.* **143,** 1008–1014.

GERN, J.E. & LEMANSKE, R.F. (1993). Beta-adrenergic agonist therapy. *Immunol. Allergy Clin. North Am.*, **13,** 839–860.

HARGREAVES, F.E., RYAN, A. & THOMPSON, N.C. (1981). Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J. Allergy Clin. Immunol.*, **68,** 347–340.

HENRIQUES, M.G. (1993). Estudo da Reação Inflamatória induzida por carragenina em camundongos. Departamento de Fisiologia e Farmacodinâmica. Fundação Oswaldo Cruz. *R.J. Brazil. PhD. Thesis*, pp. 1–181.

HENRIQUES, M.G., RAE, G., CORDEIRO, R.S.B., WILLIAMS, T.J. (1992). Endothelin-1 inhibits PAF-induced paw oedema and pleurisy in the mouse. *Br. J. Pharmacol.*, **106,** 579–582.

HOLIAN, A., HAMILTON, R. & SCHEULE, R.K. (1991). Mechanistic aspects of cromolyn sodium action on the alveolar macrophage: inhibition of stimulation by soluble agonists. *Agents Actions*, **33,** 318–325.

KEMP, J.P. (1993). Approaches to asthma management. *Arch. Intern. Med.*, **153,** 805–812.

KOWASKI, M.L., SLIWISKA-KOWASLSKI & KALINER, M.A. (1990). Neurogenic inflammation, vascular permeability and mast cells. II Additonal evidence indicating that mast cells are not involved in neurogenic inflammation. *J. Immunol.*, **145,** 1214–1221.

LO, T.N., ALMEIDA, A.P., BEAVEN, M.A. (1982). Dextran and carrageenin evoke different inflammatory responses in rat with respect to composition of infiltrates and effect of indomethacin. *J. Pharmacol. Exp. Ther.*, **221,** 261–267.

MILGROM, H. (1993). Theophylline. *Immunol. Allergy Clin. North. Am.*, **13,** 819–838.

MILGROM, H. & BENDER, B. (1993). Current Issues in the use of theophylline. *Respir. Dis.*, **147,** 533–539.

MORLEY, J.K. (1994). Channel openers and suppression of airway hyperactivity. *Trends Pharmacol. Sci.*, **15,** 463–468.

NICHOLAS, H., BLACK, P., COUCH, R., KENNEDY, J. & BRIANT, R. (1993). Theophylline target concentration in severe airway obstruction- 10 or 20 mg/L? *Clin. Pharmacocinet.*, **25,** 495–505.

O'BYRNE, P.M., DOLOVICH, J. & HARGREAVE, F.E. (1987). Late asthmatic responses. *Am. Rev. Respir. Dis.*, **136,** 740–751.

OKAYAMA, Y. & CHURCH, M.K. (1992). Comparison of the modulatory effect of ketotifen, sodium cromoglycate, procaterol and salbutamol in human skin, lung and tonsil mast cells. *Arch. Allergy Immunol.*, **97,** 316–225.

PACHECO, Y., HOSNI, R., CHABANNES, B., GORMAND, F., MOLIERE, P., GROSCLAUDE, M., PIPERNO, D., LAGARDE, M. & PERRIN-FAYOLLE, M. (1992). Leukotrien B4 level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta-2-agonist therapy. *Eur. J. Clin. Invest.*, **22,** 732–739.

PAGE, C.P. (1991). One explanation of the asthma paradox: inhibition of natural anti-inflammatory mechanism by beta-2-agonists. *Lancet*, **337,** 717–720.

PAGGIARO, P.L., DENTE, F.L., VAGAGGINI, B., BACCI, E., TALINI, D., TESTI, R., MAPP, C.E., FABBRI, L.M. & GIUNTINI, C. (1991). Salbutamol plus beclomethasone dipropionate, but not salbutamol alone, completely prevent early and late asthmatic responses to allergen. *Respir. Med.*, **85,** 401–406.

PAUWELS, R.A. (1989). New aspects of therapeutic potential of theophylline in asthma. *J. Allergy Clin. Immunol.*, **83,** 548–553.

PAUWELS, R.A. & PERSON, C.G.A. (1991). Xanthines. In *Asthma: its Pathology and Treatment*. ed. Kaliner, M.A., Barnes, P.J. & Person, C.G.A. pp. 503–521. New York: Marcel Decker.

RAEBURN, D., UNDERWOOD, S., LEWIS, S., WOODMAN, V., BATTRAM, C., TOMKINSON, A., SHARMA, S., JORDAM, R., SOUNESS, J., WEBBER, S. & KARLSSON, J. (1994). Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. *Br. J. Pharmacol.*, **113,** 1423–1431.

SCHLOSBERG, M., LIU, M.C. & BOCHNER, B.S. (1993). Pathophysiology of asthma. *Immunol. Allergy Clin. North Am.*, **13,** 721–743.

SCHRIER, D.J., LESCH, M.E., WRIGHT, C.D. & GILBERTESEN, R.S. (1990). The anti-inflammatory effects of adenosine receptor agonists on the carrageenin-induced pleural inflammatory response in rats. *J. Immunol.*, **145,** 1874–1879.

SMITH, H. (1992). Asthma, inflammation, eosinophils and bronchial hyperesponsiveness. *Clin. Exp. Allergy.*, **22,** 187–197.

SPATAFORA, M., CHIAPPARA, G., MERENDINO, A.M., D'AMICO, D., BELLIA, V. & BONSIGNORE, G. (1994). Theophylline suppresses the release of tumour necrosis factor-α by blood monocytes and alveolar macrophages. *Eur. Respir. J.*, **7,** 223–228.

SULLIVAN, P., BEKIR, S., JAFFAR, Z., PAGE, C., JEFFERY, P. & COSTELLO, J. (1994). Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet*, **343,** 1006–1008.

SVENJO, E. (1990). The hamster cheek pouch as a model in microcirculation research. *Eur. Respir. J.*, **12P:** (suppl.) 595–600.

TAINSH, K.R., LAU, H.Y., LIU, W.L. & PEARCE, F.L. (1991). The human skin mast cell: a comparison with the human lung cell and a novel mast cell type, the uterine mast cell. *Agents Actions*, **33,** 16–19.

TARAYRE, J.P., ALIAGA, M., BARBARA, M., TISSEYRE, N., VIEU, S. & TISNE-VERSAILLES, J. (1991). Pharmacological modulation of a model of bronchial inflammation after aerosol induced active anaphylactic shock in conscious guinea pigs. *Int. J. Immunopharmacol.*, **13,** 349–356.

TORPHY, T.J. & UNDEM, B.J. (1991). Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. *Thorax*, **46,** 512–523.

YAMADA, N., KADOWASKI, S. & UMEZU, S. (1992). Development of an animal model of late asthmatic response in guinea pigs and effects of anti-asthmatic drugs. *Prostaglandins*, **43,** 507–521.

YUKAWA, T., KROGEL, C., CHANEZ, P., DENT, G., UKENA, D., CHUNG, K.F. & BARNES, P.J. (1989). Effect of theophylline and adenosine on eosinophil function. *Am. Rev. Respir. Dis.*, **140,** 327–333.

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