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Local exposure to salbutamol or Bt_2 cyclic AMP inhibits pleural exudation and leukocyte influx caused by antigen in rats

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Abstract

The local effect of salbutamol and N^6,2′-*O*-dibutyryl adenosine 3′:5′-cyclic monophosphate (Bt_2 cyclic AMP) on the rat pleural inflammation caused by allergen was investigated. Antigen (ovalbumin, 12 μg/cavity) intrathoracically administered to immunized rats led to a marked pleural protein extravasation and leukocyte infiltration, as attested by the quantification of protein and enumeration of leukocytes recovered from the pleural cavity. Salbutamol (10–40 μg/cavity) and the cell-permeable cyclic AMP analogue, Bt_2 cyclic AMP (20–160 μg/cavity), injected 1 h and 5 min before the antigen, respectively, inhibited the exudation occurring within 30 min, and neutrophil and eosinophil accumulation ocurring 4 and 24 h, respectively. The late eosinophilia was also markedly attenuated by salbutamol administered 10 min post-challenge, when mast cells had already been degranulated. Pretreatment with the β-adrenoceptor antagonist propranolol (1 mg/kg, i.v.) failed to modify the inhibitory effect of Bt_2 cyclic AMP, but abolished the blockade caused by salbutamol of leukocyte infiltration under conditions where the salbutamol anti-exudatory activity was impaired to about 80%. In another set of experiments, salbutamol (20 and 40 μg/cavity) markedly inhibited the exudation caused by histamine and 5-hydroxytryptamine (5-HT) which, though to a lesser extent, was also sensitive to Bt_2 cyclic AMP (80 μg/cavity). As observed with allergic pleurisy, propranolol impaired the inhibition by salbutamol of histamine- and 5-HT-induced exudation, whereas the Bt_2 cyclic AMP inhibition was not affected. We conclude that salbutamol and Bt_2 cyclic AMP share the ability to inhibit pleural exudation and leukocyte recruitment caused by allergen in immunized rats, suggesting that the anti-inflammatory effect of salbutamol may be mediated by a cyclic AMP signaling pathway, probably via β_2-adrenoceptor activation.

Keywords: Salbutamol; Bt_2 cAMP (N^6,2′-*O*-dibutyryl adenosine 3′:5′-cyclic monophosphate); Allergic pleurisy

1. Introduction

β-Adrenoceptor agonists are used as an effective therapy for the treatment of some allergic reactions (Howarth et al., 1985; Inagaki et al., 1992). In fact, it has been demonstrated that β-adrenoceptor agonists impair some immunoglobulin (Ig) E-mediated processes including allergic rhinitis (Jorde et al., 1975), immediate hypersensitivity in human skin (Shereff et al., 1973) and passive cutaneous anaphylaxis in rats (Ankier, 1971). One explanation for the β-adrenoceptor agonist action is the inhibition of inflammatory mediator release from mast cells and basophils, which is thought to be caused by an increase in the intracellular levels of cyclic AMP (Lichtenstein and Margolis, 1968). Nevertheless, the co-administration of antigen and β-adrenoceptor agonists completely inhibits the plasma leakage in the air pouch of sensitized rats, but fails to modify the histamine levels in the pouch fluid (Ohuchi et al., 1987), indicating that the inhibition of mast cell degranulation does not fully explain the anti-inflammatory action of the β-adrenoceptor agonists. Increasing evidence indicates that this class of compounds may also directly inhibit the vascular permeability effects of some inflammatory mediators, including bradykinin and vasoactive amines (Ohuchi et al., 1990; Advenier et al., 1992). This anti-exudatory

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action of β-adrenoceptor agonists depends on the stimulation of β-adrenoceptors because their effects are susceptible to blockade by the β-adrenoceptor antagonists (Person and Erjefält, 1979; Whelan and Johnson, 1992).

In a model of allergic pleurisy established in our laboratory, we noted that antigenic challenge of immunized rats caused an inflammatory reaction that was characterized by an anaphylactic phase that was clearly dependent on vasoactive amines associated with mast cell degranulation (Lima et al., 1990). Exposure to allergen also produced cellular infiltration mainly consisting of neutrophils and eosinophils 4 and 24 h post-challenge, respectively (Lima et al., 1991). It has been demonstrated that treatment with salbutamol and salmeterol significantly blocks both the acute exudation and the eosinophil infiltration noted in a passive cutaneous anaphylaxis (PCA) reaction in rats (Teixeira et al., 1995). Nevertheless, as far as we know, there is no evidence in the literature that these phenomena can be blocked by treatment with the analogue N^6,2′-*O*-dibutyryl adenosine 3′:5′-cyclic monophosphate (Bt_2 cyclic AMP). Thus, the present study was undertaken in order to investigate the effect of the β_2-adrenoceptor agonist salbutamol and the analogue Bt_2 cyclic AMP on rat allergic pleurisy. We report here that topical treatment with salbutamol abolished the acute exudation and the late leukocyte influx, including neutrophils and eosinophils, caused by allergen. Moreover, Bt_2 cyclic AMP had the same anti-inflammatory properties, reinforcing the proposition that salbutamol requires the cyclic AMP signaling pathway to produce its effects.

2. Materials and methods

2.1. Animals and allergic pleurisy

Wistar rats of both sexes (180–200 g), from the Oswaldo Cruz Foundation breeding, were subcutaneously sensitized with a mixture of 50 μg of ovalbumin and 5 mg of $Al(OH)_3$. 14 days later, ovalbumin (12 μg/cavity) was intrathoracically (i.t.) administered to anaesthetized rats, and at 30-min to 24-h intervals the animals were killed and the thoracic cavity was washed with 3 ml of saline containing 10 IU of heparin. The volume of pleural fluid was measured with a graduated syringe. These experiments included non-sensitized rats injected i.t. with ovalbumin as negative control groups.

2.2. Pleurisy by vasoactive amines

Pleurisy was induced by the i.t. injection of histamine (200 μg/cavity) or 5-HT (100 μg/cavity), diluted with sterile 0.9% NaCl solution (saline), in a final volume of 0.1 ml. Controls received the same volume of saline. In another set of experiments, histamine (10 μg/cavity) and 5-HT (5 μg/cavity) were i.t. co-injected into normal rats and the protein extravasation was evaluated as described below.

2.3. Cell analysis

Leukocytes were counted with an automatic cell counter (Coulter model ZM) after dilution of the pleural fluid sample (40 μl) with 20 ml of Isoton II plus Zaptoglobin. The differential analysis was made in cytocentrifuged smears fixed and stained with May-Grunwald-Giemsa dye. The results were expressed as millions of cells per cavity.

2.4. Protein quantification

The fluid recovered from the pleural cavity was centrifuged for 10 min at 2000 rpm and its total protein content was quantified in the supernatant, at 540 nm, spectrophotometrically (Shimadzu Corporation) using the Biuret technique.

2.5. Histamine measurement

Histamine stored in cells recovered by washing the pleural cavity with heparinized saline was spectrofluorimetrically determined according to Shore et al., 1959. The unpurified cellular suspension (containing about 5×10^5 mast cells) was centrifuged at 300 rpm for 10 min, and 0.8 N perchloric acid (1 ml) was added to the pellet leading to cell lysis and protein precipitation. After centrifugation at $1000 \times g$ for 10 min, the supernatants were collected and stored at −20°C until histamine quantification.

2.6. Treatment

β_2-Adrenoceptor agonist salbutamol (10–40 μg/cavity) and Bt_2 cyclic AMP (20–160 μg/cavity) were diluted with sterile saline and locally administered 1 h and 5 min before the pleural challenge, respectively. The β-adrenoceptor antagonist propranolol (1 mg/kg) was i.v. administered 10 min before salbutamol or Bt_2 cyclic AMP injection. Control animals were treated only with saline.

2.7. Materials

Ovalbumin was purchased from Biochemica Fluka (Switzerland). Histamine, 5-HT, Bt_2 cyclic AMP, propranolol and *o*-phthalaldeyde, for histamine assay, were obtained from Sigma Chemical Co. (USA). Salbutamol

was kindly provided by Dr. Fernando Vieira from Glaxo do Brasil SA. All solutions were freshly prepared immediately before use.

2.8. Statistical analysis

Data are reported as means ± S.E.M. and were analysed by analysis of variance (ANOVA) followed by the Newman-Keuls-Student's *t*-test. In case of comparison between only two groups, the difference in means was analysed by unpaired Student's *t*-test. Probability values of 0.05 or less were considered significant.

3. Results

3.1. Effect of salbutamol and Bt_2 cyclic AMP on allergic pleurisy

Directly administered into the pleural cavity, 1 h before the injection of ovalbumin (12 μg/cavity), salbutamol (10–40 μg/cavity) dose dependently inhibited the increased pleural protein content (Fig. 1, left panel), evaluated 30 min after the allergen injection. In contrast, the great reduction in the stored histamine level of the pleural fluid collected 30 min post-challenge was not modified by salbutamol (40 μg/cavity) (Fig. 1, right panel). Bt_2 cyclic AMP locally injected 5 min before antigen was also able to inhibit the phenomenon though to a lesser extent. In contrast to salbutamol, the effect of Bt_2 cyclic AMP was not dose-dependent (Fig. 2).

As illustrated in Fig. 3, salbutamol (40 μg/cavity) (upper panel) and Bt_2 cyclic AMP (80 μg/cavity) (lower panel) also inhibited the pleural neutrophilia (open columns) and eosinophilia (hatched columns)

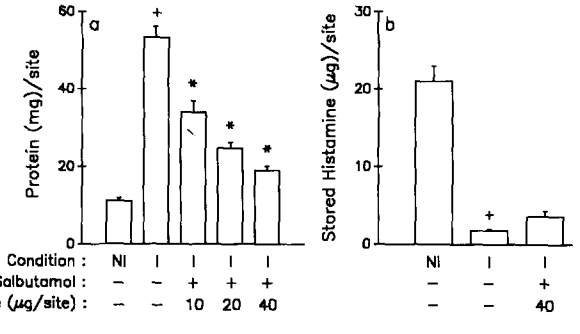

Fig. 1. Inhibition by salbutamol (10–40 μg/site) of plasma protein leakage (a) and the reduction in the stored histamine levels (b) triggered by ovalbumin (12 μg/site) in immunized (I) and non-immunized rats (NI). The analysis was made 30 min after challenge. Each column represents the mean ± S.E.M. from at least eight animals. $^{+}$ $P < 0.05$ as compared to the non-immunized group by the Newman-Keuls-Student's *t*-test; * $P < 0.05$ as compared to the immunized group by the Newman-Keuls-Student's *t*-test.

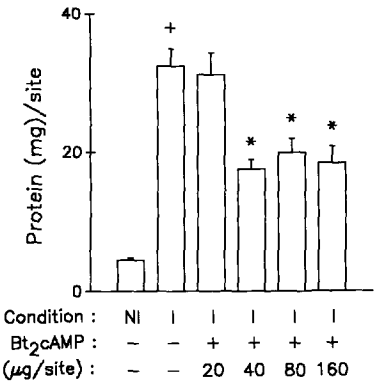

Fig. 2. Inhibition by Bt_2 cyclic AMP (20–160 μg/site) of plasma protein leakage triggered by ovalbumin (12 μg/site) in immunized (I) and non-immunized rats (NI). The analysis was made 30 min after challenge. Each column represents the mean ± S.E.M. from at least eight animals. $^{+}$ $P < 0.05$ as compared to the non-immunized group by the Newman-Keuls-Student's *t*-test; * $P < 0.05$ as compared to the immunized group by the Newman-Keuls-Student's *t*-test.

noted 4 and 24 h after antigen, respectively. It is noteworthy that neither salbutamol nor Bt_2 cyclic AMP modified the basal leukocyte population of the pleural cavity, which was $5.19 \pm 0.48 \times 10^6$ cells/cavity in naive animals, $5.36 \pm 0.59 \times 10^6$ cells/cavity and 4.83 ± 0.50 in salbutamol- and Bt_2 cyclic AMP-treated animals, respectively. In addition, local salbutamol was still ac-

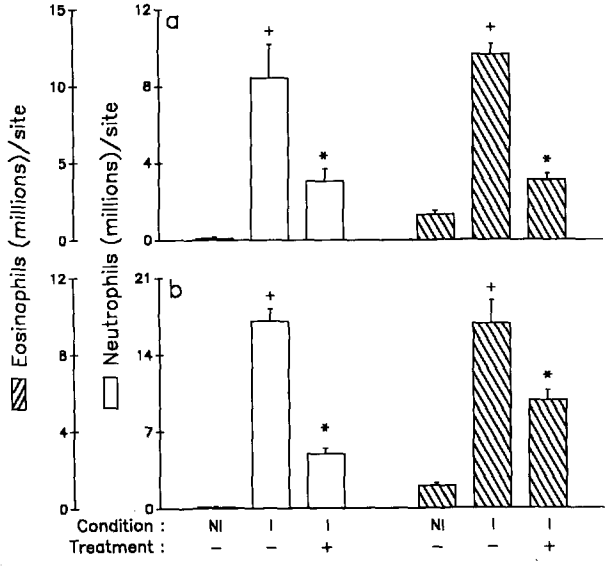

Fig. 3. Inhibition by salbutamol (40 μg/site) (a) or Bt_2 cAMP (80 μg/site) (b) of neutrophil (open columns) and eosinophil accumulation (hatched columns) triggered by ovalbumin (12 μg/site) in immunized (I) and non-immunized rats (NI). The analysis of neutrophil and eosinophil mobilization was made at 4 and 24 h after antigen stimulation, respectively. Each column represents the mean ± S.E.M. from at least eight animals. $^{+}$ $P < 0.05$ as compared to the non-immunized group by the Newman-Keuls-Student's *t*-test.; * $P < 0.05$ as compared to the immunized group by the Newman-Keuls-Student's *t*-test.

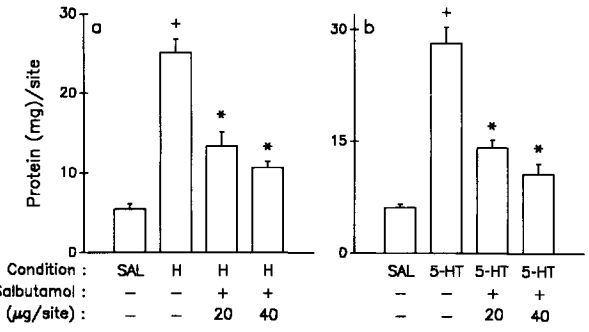

Fig. 4. Inhibition by salbutamol (20 and 40 μg/site) of protein extravasation caused by histamine (H, 200 μg/site) (a) or 5-hydroxytryptamine (5-HT, 100 μg/site) (b) in the pleural cavity of normal rats. The analysis was made 30 min after stimulation. Each column represents the mean ± S.E.M. from at least eight animals. + $P < 0.05$ as compared to the saline-injected group by the Newman-Keuls-Student's t-test; * $P < 0.05$ as compared to the respective vehicle-treated group by the Newman-Keuls-Student's t-test.

tive when administered 10 min after antigenic challenge and reduced the eosinophil number from 7.45 ± 0.88 to 3.77 ± 0.38 × 10^6 cells/cavity (means ± S.E.M.; $n = 8$, $P < 0.001$).

3.2. Effect of salbutamol and Bt_2 cyclic AMP on pleural exudation caused by vasoactive amines

Salbutamol (20 and 40 μg/cavity) i.t. injected 1 h before stimulation dose dependently abolished the reaction induced by histamine (200 μg/cavity) (Fig. 4, left panel) or 5-HT (100 μg/cavity) (Fig. 4, right panel). Again, Bt_2 cyclic AMP (80 μg/cavity) locally injected 5 min before histamine (200 μg/cavity) (Fig. 5, left panel) or 5-HT (100 μg/cavity) (Fig. 5, right panel) was also able to inhibit the pleural exudation. It is noteworthy that the sensitivity to Bt_2 cyclic AMP treatment (20–160 μg/cavity) was significantly enhanced when the reaction was triggered by a mixture of histamine and 5-HT. This combination, at doses selected to give little responses (10 and 5 μg/cavity, respectively), produced a marked synergy (Fig. 6).

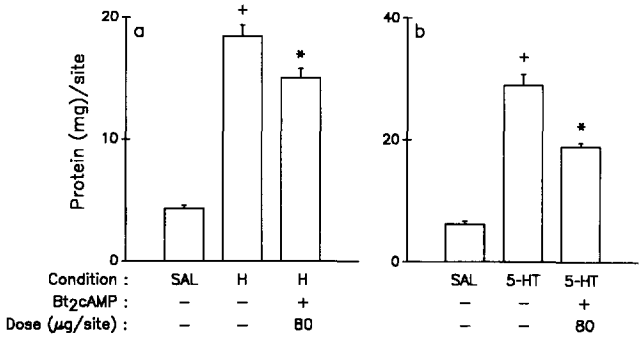

Fig. 5. Inhibition by Bt_2 cyclic AMP (80 μg/site) of protein extravasation caused by histamine (H, 200 μg/site) (a) or 5-hydroxytryptamine (5-HT, 100 μg/site) (b) in the pleural cavity of normal rats. The analysis was made 30 min after stimulation. Each column represents the mean ± S.E.M. from at least eight animals. + $P < 0.05$ as compared to the saline-injected group by the Newman-Keuls-Student's t-test; * $P < 0.05$ as compared to the respective vehicle-treated group by the Newman-Keuls-Student's t-test.

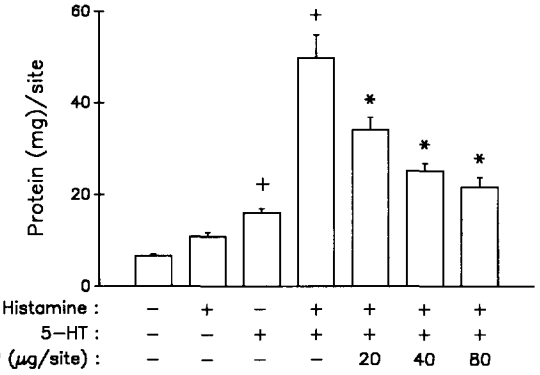

Fig. 6. Inhibition by Bt_2 cyclic AMP (20–80 μg/site) of protein extravasation caused by the co-injection of histamine (10 μg/site) and 5-hydroxytryptamine (5-HT, 5 μg/site) in the pleural cavity of normal rats. The analysis was made 30 min after stimulation. Each column represents the mean ± S.E.M. from at least eight animals. + $P < 0.05$ as compared to the saline-injected group by the Newman-Keuls-Student's t-test; * $P < 0.05$ as compared to the respective vehicle-treated group by the Newman-Keuls-Student's t-test.

3.3. Effect of propranolol on the suppressive action of salbutamol and Bt_2 cyclic AMP

As illustrated in Fig. 7, pretreatment with propranolol (1 mg/kg, i.v.) impaired the inhibitory effect of salbutamol on both neutrophil and eosinophil accumulation induced by antigen (Fig. 7b,c, respectively), under conditions where the antigen-induced exudatory response was only partially restored (80%, $P < 0.001$) (Fig. 7a). In contrast, propranolol did not alter the protective effect of Bt_2 cyclic AMP on these phenomena (Table 1).

Similarly, the inhibitory effect of salbutamol (Table 2), but not Bt_2 cyclic AMP (Table 3), on histamine- and 5-HT-induced exudation was clearly suppressed by pretreatment with propranolol.

4. Discussion

Although clinical experience has not evidenced the anti-inflammatory properties of short-acting β-adrenoceptor agonists (Archer and MacDonald, 1987; Cockroft and Murdock, 1987), several studies have documented the suppressive effect of these compounds on antigen-induced mediator release (Basran et al., 1982) and exudation and leukocyte infiltration (Person et al., 1982; Teixeira et al., 1995) in various experimental models. For example, studies of passive and active rat peritoneal anaphylaxis have emphasized the effect of

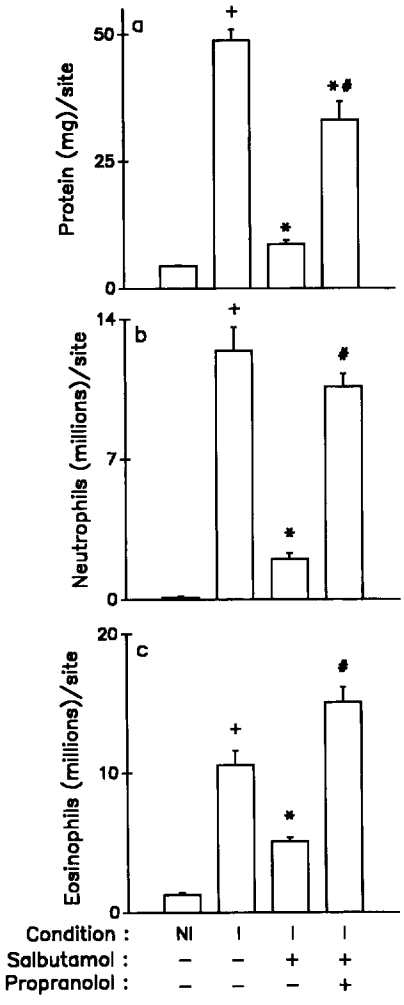

Fig. 7. Blockade by propranolol (1 mg/kg, i.v.) of the inhibitory effect of salbutamol (40 μg/site) on protein extravasation (a) neutrophil (b) and eosinophil accumulation (c) triggered by ovalbumin (12 μg/site) in immunized (I) and non-immunized rats (NI). The analysis of exudation, neutrophil and eosinophil mobilization was made at 30 min, 4 and 24 h after antigen stimulation, respectively. Each column represents the mean ± S.E.M. from at least eight animals. $^{+}$ $P < 0.05$ as compared to the non-immunized group by the Newman-Keuls-Student's t-test.; * $P < 0.05$ as compared to the immunized untreated group by the Newman-Keuls-Student's t-test.

Table 2
Effect of propranolol (1 mg/kg) on the inhibitory action of salbutamol (40 μg/site) on protein extravasation induced by histamine (200 μg/site) or 5-HT (100 μg/site) in normal rats

Stimulus	Treatment	Protein (mg/site)
Histamine	None	26.3 ± 1.0 [a]
	Salbutamol	12.0 ± 1.1 [b]
	Salbutamol + propranolol	21.4 ± 1.1 [c]
5-HT	None	56.9 ± 3.1 [a]
	Salbutamol	14.5 ± 7.0 [b]
	Salbutamol + propranolol	34.7 ± 3.8 [c]

The analysis of exudation was made at 30 min and the protein content of the pleural washing from the saline-injected rats was 7.2 ± 0.5 mg/site ($n = 7$). Values represent the means ± S.E.M. from at least eight animals. [a] $P < 0.05$ from the saline-injected group; [b] $P < 0.05$ from the stimulated non-treated group; [c] $P < 0.05$ from the salbutamol-treated stimulated group. Student's t-test was used in all conditions.

Table 3
Effect of propranolol (1 mg/kg) on the inhibitory activity of Bt_2 cyclic AMP (80 μg/site) on protein extravasation induced by histamine (200 μg/site) or 5-HT (100 μg/site) in normal rats

Stimulus	Treatment	Protein (mg/site)
Histamine	None	26.3 ± 1.0 [a]
	Bt_2 cAMP	20.5 ± 0.6 [b]
	Bt_2 cAMP + propranolol	21.6 ± 1.0
5-HT	None	56.9 ± 3.1 [a]
	Bt_2 cAMP	38.7 ± 3.3 [b]
	Bt_2 cAMP + propranolol	30.5 ± 2.9

The analysis of exudation was made in 30 min and the protein content of the pleural washing from the saline-injected rats was 6.5 ± 0.3 mg/site ($n = 8$). Values represent the means ± S.E.M. from at least eight animals. [a] $P < 0.05$ from the saline-injected group; [b] $P < 0.05$ from the stimulated non-treated group.

Table 1
Failure of propranolol (1 mg/kg) to block the inhibitory effect of Bt_2 cyclic AMP (80 μg/site) on protein extravasation, neutrophil and eosinophil accumulation triggered by antigen in immunized rats

Stimulus	Propranolol	Bt_2cAMP	Protein (mg/site)	Neutrophils (10^6 cells/site)	Eosinophils (10^6 cells/site)
OVA [a]	–	–	5.4 ± 1.2	0 ± 0	1.9 ± 0.3
OVA [b]	–	–	53.3 ± 1.1 [c]	7.1 ± 1.1 [c]	9.3 ± 0.5 [c]
OVA [b]	–	+	25.1 ± 1.1 [d]	3.1 ± 0.7 [d]	6.0 ± 0.5 [d]
OVA [b]	+	+	22.4 ± 2.6 [d]	2.2 ± 0.4 [d]	6.1 ± 0.8 [d]

The analysis of exudation, neutrophil and eosinophil mobilization was made at 30 min, 4 and 24 h after ovalbumin (OVA) (12 μg/site) injection into immunized [b] and non-immunized [a] rats. Values represent the means ± S.E.M. from at least eight animals. [c] $P < 0.05$ as compared to non-immunized group by the Newman-Keuls-Student's t-test; [d] $P < 0.05$ as compared to immunized the untreated group by the Newman-Keuls-Student's t-test.

both isoprenaline and salbutamol on exudation and neutrophil mobilization triggered by antigen (Sharpe and Smith, 1979; Orange et al., 1970). It has been largely accepted that the mechanism of anti-allergic action of β-adrenoceptor agonists is dependent on the elevation of intracellular levels of cyclic AMP, leading to mediator release down-regulation, as suggested by in vitro observations (Orange et al., 1970; Orange et al., 1971). Nevertheless, as far as we know, there is no evidence in the literature indicating that cyclic AMP or its analogues can affect allergic inflammatory responses in vivo.

In the present study, the mode of action of short-acting β_2-adrenoceptor agonists was investigated on the potential effectiveness of salbutamol and of the cell-permeable analogue Bt_2 cyclic AMP on the allergic pleurisy triggered by antigen in immunized rats. We verified that the acute exudatory response noted 30 min after antigen challenge was markedly inhibited by the local administration of salbutamol, under conditions where the concomitant reduction in the content of histamine stored in mast cells harvested from the pleural cavity was not modified by such treatment. These findings are in line with those provided by Ohuchi et al. (Ohuchi et al., 1987, 1990), who with an air pouch model of allergic inflammation in rats failed to demonstrate a correlation between inhibition of the vascular permeability and blockade of histamine release, following treatment with either isoprenaline or salbutamol. In addition, procaterol inhibited plasma leakage without interfering with mast cell degranulation (Ohuchi et al., 1987), indicating that the effect of the β-adrenoceptor agonists on exudation induced by antigen appears to be indeed independent of the recognized capacity of these drugs to inhibit the release of mast cell derived inflammatory mediators.

We have demonstrated that antigen-induced rat pleural exudation is clearly dependent on histamine and 5-HT (Lima et al., 1991). Since the inhibition of histamine release does not seem to account for the anti-inflammatory effect of salbutamol, we investigated the ability of this drug to directly antagonize the pleural permeability response caused by vasoactive amines in naive rats. Similarly to what was observed in the case of antigen-induced exudation, the local treatment with salbutamol significantly abolished, though to a lesser extent, the protein extravasation triggered by histamine and 5-HT. These results confirmed previous data, in which the β-adrenoceptor agonists directly inhibited protein extravasation caused by histamine, bradykinin and other agonists in models of guinea pig airway inflammation and rat air pouch (Ohuchi et al., 1990; Advenier et al., 1992). Moreover, they are consistent with the interpretation that the anti-exudatory effect of β-adrenoceptor agonists may result from a direct action upon the endothelial microvasculature.

As previously mentioned, it has been hypothesized that the anti-inflammatory effect of β-adrenoceptor agonists – similarly to their ability to induce relaxation of airway smooth muscle (Howarth et al., 1985) – results from an elevation of intracellular levels of cyclic AMP, which is supposed to regulate cell functions negatively (Pessin et al., 1983). Thus, using a cell-permeable analogue Bt_2 cyclic AMP, which penetrates the cell membrane better (Thivierge et al., 1993), we tried to mimic the protective action of salbutamol on the anaphylactic phase of allergic pleurisy. Locally administered 5 min before pleural challenge, Bt_2 cyclic AMP significantly prevented the protein leakage caused by antigen, but in contrast to salbutamol, the blockade was not dose-dependent. One possible explanation for the absence of a dose-dependent inhibition may be the saturation of intracellular enzymes responsible for cleavage of the acyl group of cyclic AMP derivatives, a mechanism important for their biological activity (Henion et al., 1967). Pleural protein leakage appeared to be partially inhibited by Bt_2 cyclic AMP, never reduced more than 55%, an effect probably dependent on the complexicity of the allergen-induced inflammatory reaction. Although to a lesser extent, the exudation caused by histamine and 5-HT was also inhibited by Bt_2 cyclic AMP. It is noteworthy that sensitivity to this compound was more pronounced when a mixture of subliminal doses of histamine and 5-HT was used as challenge, which caused a clear synergistic exudatory response. Consequently, it is not unlikely that the anti-vasopermeation action of salbutamol is mediated by a cyclic AMP signaling pathway.

In vivo studies have demonstrated that salbutamol is able to inhibit neutrophil accumulation triggered by either antigen or zymosan (Whelan and Johnson, 1992; Sharpe and Smith, 1979; Hutson et al., 1988). However, though this drug was shown to be inactive against eosinophil recruitment (Tarayre et al., 1991), Teixeira et al. (1995) have recently demonstrated that salbutamol when given concomitantly or 30 min before the challenge markedly inhibited the eosinophil infiltration noted 2 h later. Here we have demonstrated that local treatment with salbutamol did not alter the basal levels of pleural leukocytes, but markedly inhibited both neutrophil and eosinophil accumulation noted 4 and 24 h after antigen stimulation, respectively. The apparent discrepancy between our findings and others, in regard to the effectiveness of salbutamol in impairing the late eosinophil accumulation, may possibly reflect differences in the kinetics and/or the mechanism of eosinophil influx into the pleural cavity, compared to what occurs in other experimental conditions. It is of interest to point out that the anti-eosinophilotactic effect of salbutamol was also noted when its administration occurred 10 min post-challenge – under conditions when mast cells had already been completely

degranulated – which would be indicative that both phenomena are indeed dissociated. Since the allergic pleural eosinophilia seen at 24 h was previously shown to be dependent on eicosanoids and platelet activating factor (PAF) (Silva et al., 1992), one possible explanation for this inhibitory effect is that salbutamol may be acting on an intermediate step of eosinophil recruitment, probably by blocking the generation and/or release of some eosinophil chemotactic factors not preformed in the mast cell granules. Alternatively, salbutamol may be acting in the post-capillary venules, since it is at this level that the cells migrate from the vascular to the tissue compartment (Hurley, 1978). As with salbutamol, the local administration of Bt_2 cyclic AMP also did not modify the basal leukocyte population of the pleural cavity, under conditions where it was effective in preventing both neutrophil and eosinophil accumulation triggered by antigen.

As expected, the anti-inflammatory action of the β-adrenoceptor agonists appears to be markedly dependent on the stimulation of β-adrenoceptors, since this effect is, in general, completely abolished by propranolol (Sharpe and Smith, 1979; Green, 1972). However, it was reported that the blockade by salmeterol and formoterol of the secretion of eicosanoids and interleukin 1 from zymosan-stimulated monocyte cultures was not antagonized by treatment with the β-adrenoceptor antagonist sotalol (Linden, 1992), suggesting that β_2-adrenoceptor agonist effects may not be exclusively accounted for by an action on β-adrenoceptors. The i.v. treatment with propranolol failed to modify the antigen-induced leukocyte infiltration, under conditions where it clearly impaired the inhibitory effect of salbutamol on both neutrophil and eosinophil infiltration. However, the protective activity of salbutamol on the plasma leakage triggered by antigen, histamine or 5-HT was significantly inhibited, but not abolished, by the β-adrenoceptor antagonist, which indicates that the anti-exudatory effect of salbutamol is not entirely mediated by β_2-adrenoceptors. In contrast, the inhibitory effect of Bt_2 cyclic AMP on exudation and leukocyte infiltration, including neutrophils and eosinophils, was not affected by propranolol, adding support to the concept that Bt_2 cyclic AMP is indeed acting on a post-receptor step in the cell signaling process.

In conclusion, our findings indicate that the plasma protein leakage and leukocyte infiltration triggered by antigen in immunized rats are both sensitive to local administration of salbutamol. These effects are mainly mediated by β_2-adrenoceptors and appear to be independent of the hypothesized protective activity on mast cell histamine release. In addition, since salbutamol and Bt_2 cyclic AMP share a similar profile of anti-inflammatory properties, it is quite possible that the β_2-adrenoceptor agonists are indeed acting via a cyclic AMP signaling pathway.

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