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# Anti-inflammatory effects of a short-acting and a long-acting $\beta_2$-adrenoceptor agonist in guinea pig skin

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**Abstract**

The pharmacological modulation of the accumulation and function of eosinophils in tissues may have a significant impact in the treatment of allergic diseases such as asthma, atopic dermatitis and rhinitis. In this study, we have investigated the acute anti-inflammatory effects of a short-acting (salbutamol) and a long-acting (salmeterol) $\beta_2$-adrenoceptor agonist on $^{111}$In-accumulation and oedema formation in allergic and mediator-induced inflammation in guinea pig skin. Both salbutamol and salmeterol inhibited $^{111}$In-eosinophil accumulation induced by platelet-activating factor and in a passive cutaneous anaphylactic reaction when co-injected with the inflammatory stimuli or when given as a 30 min pretreatment. The inhibition was reversed by DL-propranolol, but not D-propranolol. Systemic treatment with salbutamol inhibited $^{111}$In-eosinophil accumulation and oedema formation when given as a 15 min, but not as a 3 h, pretreatment. In contrast, salmeterol was effective when given at both times. We conclude that a long duration of action of $\beta_2$-adrenoceptor agonists is not necessary to demonstrate acute anti-inflammatory effects on eosinophil accumulation in guinea pig skin.

*Keywords:* Eosinophil; $\beta$-Adrenoceptor agonist; Allergy; Inflammation; Passive cutaneous anaphylactic reaction

## 1. Introduction

Eosinophils are thought to play an important role in the pathophysiology of various allergic diseases such as asthma (Venge and Hakansson, 1991), atopic dermatitis (Bruijnzeel-Koomen et al., 1992) and rhinitis (Klementsson, 1992). Thus, drugs which inhibit the accumulation of these cells into sites of inflammation may be useful in the treatment of allergic diseases.

In guinea pig skin, radiolabelled eosinophils accumulate in a passive cutaneous anaphylactic reaction (type I hypersensitivity) and in response to i.d. injection of different mediators of inflammation (Faccioli et al., 1991; Teixeira et al., 1993a). This accumulation of radiolabelled eosinophils is dependent on the expression of both CD18 (Teixeira et al., 1994a) and very late activation antigen-4 (VLA-4; Weg et al., 1993) integrins on the eosinophil surface and can be modulated by different pharmacological agents (Teixeira and Hellewell, 1993; Teixeira et al., 1993a,b). Of interest, local administration of drugs which enhance cyclic adenosine monophosphate (cyclic AMP) levels within different cells effectively inhibit eosinophil, but not neutrophil, accumulation in skin in response to various inflammatory stimuli (Teixeira et al., 1993a, 1994b).

There is much evidence indicating that $\beta$-adrenoceptors are linked to adenylate cyclase and their occupation leads to elevation of cyclic AMP in different cell types (Goldie et al., 1991). The presence and functional characteristics of $\beta$-adrenoceptors on human and guinea pig eosinophils has been investigated (Kita et al., 1991; Munoz et al., 1994; Yukawa et al., 1990). These cells possess $\beta$-adrenoceptor receptors of the $\beta_2$-subtype which are linked to adenylate cyclase as assessed by their capacity, following occupation with specific agonists, to increase intracellular cyclic AMP levels (Kita et al., 1991; Yukawa et al., 1990). Activation of the receptor with $\beta$-adrenoceptor agonists inhibits eosinophil degranulation, leukotriene $C_4$ production and chemotaxis (Kita et al., 1991; Koenderman et al., 1992; Munoz et al., 1994).

Studies in experimental animals (Whelan and Johnson, 1992; Whelan et al., 1993) have shown acute

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inhibitory effects of the long-acting $\beta_2$-adrenoceptor agonist, salmeterol, on granulocyte accumulation. The situation regarding short-acting $\beta_2$-adrenoceptor agonist is less clear since some authors, but not others, failed to show inhibition of eosinophil accumulation by these drugs in different animal models. For example, eosinophil accumulation in guinea pig lung measured 24 or 48 h after aerosolized platelet-activating factor (PAF) was not inhibited by a 30 min pretreatment or continuous treatment with salbutamol (Sanjar et al., 1990; Whelan and Johnson, 1992). In contrast, co-administration of isoprenaline significantly inhibited blood and lung eosinophilia following intravenous sephadex injection in rats (Spicer et al., 1990). Similarly, terbutaline, a short-acting $\beta_2$-adrenoceptor agonist, effectively inhibited antigen-induced eosinophil accumulation in human skin (Ting et al., 1983). We are not aware of any published evidence for an inhibitory effect of salbutamol on eosinophil migration in vivo. In the present study, we compared the anti-inflammatory effects of a short-acting (salbutamol) and a long-acting (salmeterol) $\beta_2$-adrenoceptor agonist, administered locally or systemically, on oedema formation and $^{111}$In-eosinophil accumulation in allergic and non-allergic inflammation in guinea pig skin.

## 2. Materials and methods

### 2.1. Preparation of zymosan-activated plasma

Zymosan-activated plasma was used as a source of guinea pig complement fragment C5a des-Arg. Zymosan-activated plasma was prepared by incubating heparinized (10 IU/ml) plasma obtained from naive guinea pigs (Harlan Porcellus, Oxon, 350–400 g) with zymosan (5 mg/ml) for 30 min at 37°C. Zymosan was then removed by centrifugation ($2 \times 10$ min at $3000 \times g$). The activated plasma was desalted using a PD-10 Sephadex G-25M column and stored in aliquots at −20°C.

### 2.2. Preparation of passive cutaneous anaphylactic sera and reactions

Details of the preparation of $IgG_1$-rich sera are described elsewhere (Weg et al., 1991). Briefly, male guinea pigs (Harlan Porcellus, 350–400 g) were immunized with bovine γ-globulin in Freund's complete adjuvant (0.2 mg bovine γ-globulin/0.2 ml of adjuvant s.c.). These animals received a boost of antigen in Freund's incomplete adjuvant on day 21 and the serum was prepared on day 30. Recipient animals received an injection of 50 μl of a 1/50 dilution of the anti-serum i.d., followed 16–20 h later by the i.d. injection of antigen (bovine γ-globulin, 0.01–1.0 μg per site).

### 2.3. Measurement of local oedema formation and $^{111}$In-eosinophil accumulation in guinea pig skin

Radiolabelled eosinophil infiltration and oedema formation at skin sites were measured simultaneously. $^{125}$I-human serum albumin (~5 μCi) was added to $^{111}$In-labelled eosinophils (purified and radiolabelled as previously described; Faccioli et al., 1991; Teixeira et al., 1994b) and injected i.v. ($2.5 \times 10^6$ cells per animal) into recipient guinea pigs (350–400 g) anaesthetized with Hypnorm (0.15 ml i.m.). After 5 min, duplicate i.d. injections of inflammatory stimuli or antigen were given in 0.1 ml volumes into the dorsal shaved skin following a randomized injection plan. For local treatment, salbutamol or salmeterol were mixed with the stimuli or saline before the i.d. injections. In some experiments, salbutamol or salmeterol were administered as a 30 min pretreatment. Propranolol was given as a co-injection with inflammatory stimuli and salbutamol or salmeterol. For systemic treatment, salbutamol or salmeterol were injected i.v. at the dose of 0.1 mg/kg, 15 min or 3 h prior to the i.v. injection of radiolabelled eosinophils and $^{125}$I-human serum albumin. Similar doses of salbutamol have been previously used in guinea pigs (Boschetto et al., 1989). Inflammatory responses ($^{111}$In-labelled eosinophil accumulation and oedema formation) were assessed 2 h after i.d. injections. This time was chosen based on previous experiments carried out in our laboratory which indicated that most $^{111}$In-eosinophil accumulation and oedema formation occur over the first 60 and 30 min, respectively, following the i.d. injection of inflammatory stimuli and in the passive cutaneous anaphylactic reaction (Weg et al., 1992; Teixeira et al., 1993c; Collins et al., 1993). At this time, a blood sample was obtained by cardiac puncture, the animals were killed with an overdose of sodium pentobarbitone, the dorsal skin was removed, cleaned free of excess blood and the sites punched out with a 17 mm punch. The samples were counted in an automatic 5-head γ-counter (Canberra Packard, Pangbourne, Berks, UK) and the counts were cross-channel corrected for the two isotopes.

Eosinophil numbers in the skin sites were expressed as the number of $^{111}$In-eosinophils per skin site and oedema formation as the ratio of $^{125}$I counts of the skin sample divided by the $^{125}$I counts in 1 μl of plasma.

### 2.4. Reagents

The following compounds were purchased from Sigma Chemical Company (Poole, Dorset): bradykinin, bovine γ-globulin, dimethyl sulphoxide (DMSO), histamine, DL-propranolol, D-propranolol and zymosan. Hanks solutions, Hepes buffer and horse serum were purchased from Life Technologies (Paisley, Scotland). Percoll was purchased from Pharmacia (Milton Keynes,

Bucks), PAF (C16) from Bachem (Saffron Walden, Essex) and leukotriene $B_4$ from Cascade Biochem (Reading, Berks). $^{125}$I-human serum albumin and $^{111}InCl_3$ were obtained from Amersham International (Amersham, Bucks). Salmeterol base (Ball et al., 1991) was synthesized and kindly supplied by Ciba Geigy, Switzerland as a racemic mixture of *R* and *S* isomers. Salmeterol was dissolved in 100% DMSO and diluted further in saline. The concentration of DMSO in the injection of fluid was 1% or smaller in all experiments. Salbutamol sulphate was purchased from Allen& Hanburys, Uxbridge, Middlesex.

### 2.5. Statistics

Experiments were analyzed by using two-way analysis of variance (ANOVA) on normally distributed data. *P* values were assigned using the Newman-Keuls procedure and values of $P < 0.05$ were considered statistically significant. Percentage inhibition was calculated after subtracting background values. Results are presented as the means ± S.E.M.

## 3. Results

The guinea pig peritoneal eosinophils used in this study had a purity of 96.5 ± 0.5% ($n = 16$ preparations). The main contaminating cells were mononuclear with occasional neutrophils. Two h after i.v. injection, over 9% of the total $^{111}$In-eosinophils injected were circulating and over 93% of total plasma $^{111}$In was bound to these cells. Systemic treatment with salbutamol or salmeterol had no effect on the number of circulating radiolabelled eosinophils or on the amount of $^{111}$In bound to the circulating cells (data not shown).

### 3.1. Effects of i.d. injection of salbutamol

I.d. injection of salbutamol ($9 \times 10^{-11}$ to $9 \times 10^{-9}$ mol per site) with stimuli led to an apparent dose-dependent inhibition of $^{111}$In-eosinophil accumulation in the passive cutaneous anaphylactic reaction and in response to i.d. injection of PAF, although this was only significant at the top dose of salbutamol tested (Fig. 1a). Oedema formation in the passive cutaneous anaphylactic reaction was also slightly, but significantly, inhibited when salbutamol was co-injected at $9 \times 10^{-9}$ mol per site with antigen (Fig. 1b). Local injection of salbutamol had no effect on oedema formation in response to i.d. injection of PAF (Fig. 1b) or histamine ($2.5 \times 10^{-8}$mol per site, data not shown).

### 3.2. Effects of i.d. injection of salmeterol

Local injection of salmeterol with antigen also inhibited $^{111}$In-eosinophil accumulation in the passive cutaneous anaphylactic reaction: passive cutaneous anaphylactic reaction 1 μg of bovine γ-globulin per site, 3813 ± 1433 $^{111}$In-eosinophils; passive cutaneous anaphylactic reaction + salmeterol $10^{-7}$ mol per site, 2369 ± 703 ($P < 0.05$, $n = 3$); saline, 501 ± 16. Oedema formation measured in the same sites was also inhibited: passive cutaneous anaphylactic reaction, 28.8 ± 3.6 μl; passive cutaneous anaphylactic reaction + salmeterol, 18.8 ± 2.2 μl ($P < 0.05$); saline, 8.9 ± 1.4 μl. Lower doses of salmeterol ($10^{-9}$ and $10^{-8}$ mol per site) had no significant effect on inflammatory responses in the passive cutaneous anaphylactic reaction (data not shown). Salmeterol ($10^{-7}$ mol per site) significantly inhibited $^{111}$In-eosinophil accumulation induced by PAF (Fig. 2b).

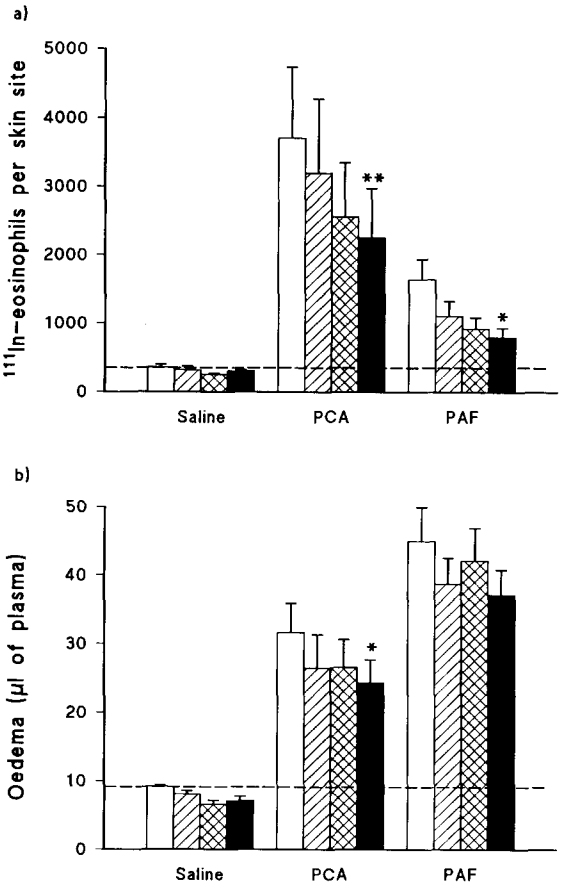


Fig. 1. Effect of i.d. injection of salbutamol on $^{111}$In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylactic (PCA) reaction and in response to PAF. Inflammatory responses were assessed 2 h after i.d. injection of saline, PAF ($10^{-9}$mol per site) or antigen (bovine γ-globulin, 1 μg per site) in sites previously sensitized with $IgG_1$-rich anti-sera either in the absence (open bars) or in the presence of salbutamol $9 \times 10^{-11}$ mol per site (hatched bars), $9 \times 10^{-10}$ mol per site (cross-hatched bars) or $9 \times 10^{-9}$ mol per site (solid bars). The line across the graphs represents background values obtained in response to i.d. injection of saline. Results are means ± S.E.M. of five guinea pigs where * and ** represent $P < 0.05$ and $P < 0.01$, respectively, when compared to responses with stimuli alone (open bars).

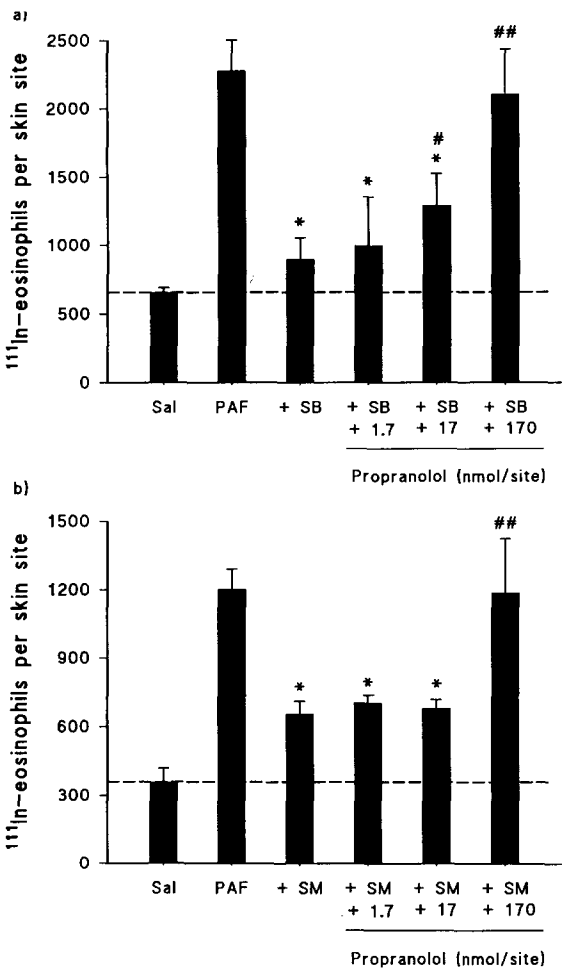


Fig. 2. Effect of i.d. injection of DL-propranolol on the inhibition of PAF-induced $^{111}$In-eosinophil accumulation by salbutamol (a) or salmeterol (b). PAF ($10^{-9}$mol per site) was injected alone, with salbutamol ($9\times10^{-9}$ mol per site) or salmeterol ($10^{-7}$ mol per site), or with either drug and increasing concentrations of DL-propranolol ($1.7\times10^{-9}$ to $1.7\times10^{-7}$ mol per site). The line across the graph represents background values obtained in response to i.d. injection of saline. Results are means ± S.E.M. of four guinea pigs where * represents $P < 0.01$ when compared to PAF alone and # and ## represent $P < 0.05$ and $P < 0.01$, respectively, when compared to PAF + salbutamol/salmeterol.

### 3.3. Effects of local pretreatment with salbutamol or salmeterol

Thirty min pretreatment of skin sites with $10^{-8}$ mol/site salmeterol or $10^{-8}$ mol/site salbutamol prior to i.d. injection of PAF ($10^{-9}$ mol/site) significantly inhibited PAF-induced $^{111}$In-eosinophil accumulation by 63% and 50%, respectively ($P < 0.05$, $n = 4$ animals). Smaller doses ($10^{-10}$ and $10^{-9}$ mol/site) of salmeterol or salbutamol had no significant effect on PAF-induced $^{111}$In-eosinophil accumulation (data not shown). In the same experiments, co-injection of salbutamol ($10^{-8}$ mol/site), but not of salmeterol ($10^{-8}$ mol/site), significantly inhibited PAF ($10^{-9}$ mol/site)-induced $^{111}$In-eosinophil accumulation by 67% ($P < 0.01$, $n = 4$ animals).

### 3.4. Effects of β-blockade

In order to assess whether the inhibitory effect of salbutamol on PAF-induced $^{111}$In-eosinophil accumulation was mediated by β-adrenoceptors, animals received i.d. injections of PAF and salbutamol and increasing concentrations of DL-propranolol ($1.7\times10^{-9}$ to $1.7\times10^{-7}$ mol per site). As shown in Fig. 2a, co-injection of DL-propranolol effectively reversed the inhibitory effect of salbutamol on PAF-induced $^{111}$In-eosinophil accumulation. In further experiments, we found that only DL-propranolol, and not D-propranolol (which has little β-adrenergic blocking activity), effectively reversed the inhibition by salbutamol of PAF-induced $^{111}$In-eosinophil accumulation (Table 1) suggesting that the reversal was a consequence of blocking β-adrenoceptors. Neither DL-propranolol, nor D-propranolol alone had any significant effect on PAF-induced $^{111}$In-eosinophil accumulation (Table 1). Co-injection of DL-propranolol ($1.7\times10^{-7}$ mol per site) with salmeterol also completely reversed the inhibitory effects of salmeterol on PAF-induced $^{111}$In-eosinophil accumulation (Fig. 2b).

### 3.5. Effects of systemic treatment with salbutamol

Fig. 3 shows the effects of systemic administration of salbutamol (0.1 mg/kg, i.v.) given 15 min prior to the experiment (salbutamol 15 min group) on $^{111}$In-eosinophil accumulation and oedema formation in the passive cutaneous anaphylactic reaction. Both parameters measured were significantly inhibited by salbutamol. In addition, in the salbutamol 15 min group, both

Table 1
Effect of DL-propranolol and D-propranolol on the inhibition by salbutamol of PAF-induced $^{111}$In-eosinophil accumulation

| | Control | $^{111}$In-eosinophils per site | | | |
|---|---|---|---|---|---|
| | | DL-propranolol | | D-propranolol | |
| | | $1.7\times10^{-8}$ | $1.7\times10^{-7}$ | $1.7\times10^{-8}$ | $1.7\times10^{-7}$ |
| Saline | 212 ± 34 | 236 ± 30 | 258 ± 46 | 214 ± 252 | 231 ± 28 |
| PAF $10^{-9}$ | 1610 ± 492 | 1744 ± 706 | 1646 ± 484 | 1194 ± 252 | 1531 ± 230 |
| PAF + SB $9\times10^{-9}$ | 288 ± 24 [a] | 586 ± 135 [b] | 1260 ± 326 [b] | 360 ± 72 | 653 ± 53 [b] |

Doses of PAF, salbutamol (SB), D-propranolol and DL-propranolol are given in mol per site. Inflammatory stimuli were given i.d. and $^{111}$In-eosinophil accumulation assessed after 2 h. Results are means ± S.E.M. of four animals; [a] $P < 0.01$ when compared to sites injected with PAF alone and [b] $P < 0.01$ when compared to sites injected with PAF and salbutamol.

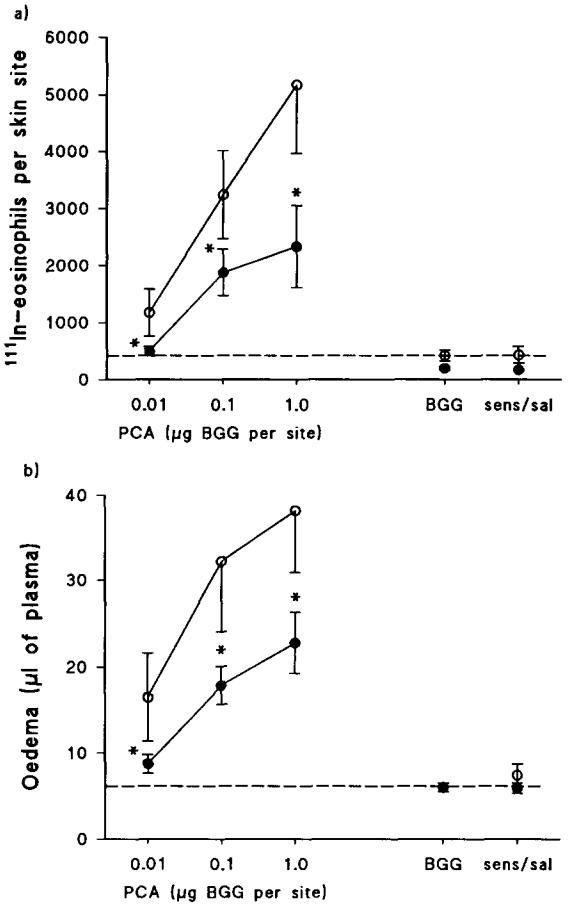


Fig. 3. Effect of systemic treatment with salbutamol (0.1 mg/kg, 15 min prior to i.v. injection of radiolabelled cells and albumin) on $^{111}$In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylactic (PCA) reaction. Control animals are shown by open circles and treated animals by filled circles. Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine γ-globulin) in saline-treated sites (shown as BGG) or sites previously sensitized with $IgG_1$-rich anti-sera (shown as PCA). The lines across the graphs represent background values obtained in sensitized skin sites injected with saline (sens/sal) in control (dashed line) and salbutamol-treated (dotted line) animals. Results are means ± S.E.M. of six pairs of guinea pigs where * represents $P < 0.05$ when compared to control animals.

oedema formation and $^{111}$In-eosinophil accumulation in response to i.d. injection of PAF ($10^{-10}$ and $10^{-9}$ mol per site), zymosan-activated plasma (10 and 30% in saline) and histamine ($2.5 \times 10^{-9}$ and $2.5 \times 10^{-8}$ mol per site) were significantly inhibited when compared to controls (Table 2). In preliminary experiments, lower doses of salbutamol (0.01 mg/kg, i.v.) given as a 15 min pretreatment also inhibited $^{111}$In-eosinophil accumulation and oedema formation in the passive cutaneous anaphylactic reaction but was less effective than 0.1 mg/kg (data not shown).

In contrast to the effects described above, systemic treatment with salbutamol (0.1 mg/kg, i.v.) 3 h prior to i.v. injection of radiolabelled eosinophils and $^{125}$I-human serum albumin had no effect on $^{111}$In-eosinophil accumulation or oedema formation in the passive cutaneous anaphylactic reaction except for a small inhibition of $^{111}$In-eosinophil accumulation at 1.0 μg of bovine γ-globulin (Fig. 4). Responses to injection of PAF, zymosan-activated plasma and histamine were also unaffected by 3 h pretreatment with salbutamol (data not shown).

### 3.6. Effects of systemic treatment with salmeterol

Salmeterol (0.1 mg/kg, i.v.) effectively inhibited $^{111}$In-eosinophil accumulation in the passive cutaneous anaphylactic reaction when given either 15 min or 3 h prior to i.v. injection of radiolabelled eosinophils and $^{125}$I-human serum albumin (Fig. 5a). There was no significant difference between the degree of inhibition of $^{111}$In-eosinophil accumulation between the different times chosen for treatment (Fig. 5a). Similarly, oedema formation was significantly inhibited when salmeterol was given 15 min or 3 h prior to the experiment (Fig. 5b). Salmeterol also inhibited $^{111}$In-eosinophil accumulation in response to i.d. injection of PAF ($10^{-10}$ and $10^{-9}$ mol per site), histamine ($2.5 \times 10^{-9}$ and $2.5 \times 10^{-8}$ mol per site) and zymosan-activated plasma (10% in saline) when administered as either a 15 min or 3 h

Table 2
Effect of 15 min systemic pretreatment with salbutamol (0.1 mg/kg, i.v.) on mediator-induced inflammation in guinea pig skin

| | | $^{111}$In-eosinophils per site | | Oedema (μl of plasma) | |
|---|---|---|---|---|---|
| | | Control | Salbutamol | Control | Salbutamol |
| PAF | $10^{-10}$ | 1499 ± 262 | 540 ± 91 [b] | 39.9 ± 5.3 | 26.7 ± 2.6 [a] |
| | $10^{-9}$ | 3030 ± 804 | 996 ± 222 [b] | 62.2 ± 5.6 | 41.9 ± 5.5 [b] |
| ZAP | 10% | 2845 ± 524 | 1352 ± 356 [b] | 33.8 ± 3.8 | 19.1 ± 2.0 [b] |
| | 30% | 7414 ± 1414 | 3911 ± 900 [b] | 37.2 ± 5.2 | 24.8 ± 3.1 [b] |
| | 100% | 17845 ± 3568 | 11855 ± 2476 | 38.7 ± 3.3 | 19.6 ± 3.1 [b] |
| Hist | $2.5 \times 10^{-9}$ | 751 ± 139 | 327 ± 49 [b] | 36.5 ± 3.6 | 23.2 ± 2.9 [b] |
| | $2.5 \times 10^{-8}$ | 744 ± 126 | 306 ± 35 [b] | 55.8 ± 8.2 | 38.4 ± 3.2 [a] |
| Saline | | 278 ± 36 | 199 ± 22 | 8.1 ± 0.9 | 6.2 ± 1.3 |

Doses of PAF and histamine (Hist) are given in mol per site and doses of zymosan-activated plasma (ZAP) as % dilution in saline. Inflammatory stimuli were given i.d. and $^{111}$In-eosinophil accumulation and oedema formation assessed after 2 h. Results are means ± S.E.M. of six pairs of animals; [a] $P < 0.05$ and [b] $P < 0.01$ when compared to control.

Table 3
Effect of systemic treatment with salmeterol (0.1 mg/kg, i.v.) on mediator-induced $^{111}$In-eosinophil accumulation in guinea pig skin

| | | $^{111}$In-eosinophils per site | | | Oedema (μl of plasma) | | |
|---|---|---|---|---|---|---|---|
| | | Control | Salmeterol 15 min | Salmeterol 3 h | Control | Salmeterol 15 min | Salmeterol 3 h |
| PAF | $10^{-10}$ | 1966 ± 324 | 598 ± 171 [a] | 1000 ± 162 [a] | 36.4 ± 6.1 | 27.7 ± 4.6 | 33.8 ± 3.5 |
| | $10^{-9}$ | 3712 ± 563 | 980 ± 255 [a] | 1819 ± 341 [a] | 61.1 ± 12.2 | 51.3 ± 3.8 | 57.9 ± 6.2 |
| ZAP | 10% | 2379 ± 273 | 1025 ± 192 [a] | 1275 ± 277 [a] | 32.4 ± 8.0 | 16.8 ± 2.9 [b] | 23.3 ± 3.7 |
| | 30% | 5205 ± 441 | 3420 ± 535 | 3616 ± 763 | 37.6 ± 7.6 | 21.9 ± 2.9 [b] | 33.9 ± 2.5 |
| | 100% | 13260 ± 2221 | 12350 ± 3039 | 8928 ± 1532 | 46.2 ± 9.4 | 36.4 ± 5.8 | 45.5 ± 5.4 |
| Hist | $2.5 \times 10^{-9}$ | 551 ± 82 | 257 ± 71 [a] | 268 ± 36 [a] | 37.9 ± 10.3 | 18.3 ± 2.5 [b] | 24.1 ± 4.1 [a] |
| | $2.5 \times 10^{-8}$ | 958 ± 248 | 177 ± 51 [a] | 390 ± 80 [a] | 60.8 ± 15.2 | 38.7 ± 5.9 [a] | 49.2 ± 4.6 |
| Saline | | 343 ± 56 | 242 ± 87 | 229 ± 62 | 9.7 ± 2.0 | 7.3 ± 1.0 | 7.1 ± 1.2 |

Salmeterol was administered i.v. either 15 min or 3 h prior to inducing inflammation. Doses of PAF and histamine (Hist) are given in mol per site and doses of zymosan-activated plasma (ZAP) as % dilution in saline. Inflammatory stimuli were given i.d. and $^{111}$In-eosinophil accumulation assessed after 2 h. Results are means ± S.E.M. of five pairs of animals; [a] $P < 0.05$ and [b] $P < 0.01$ when compared to control.

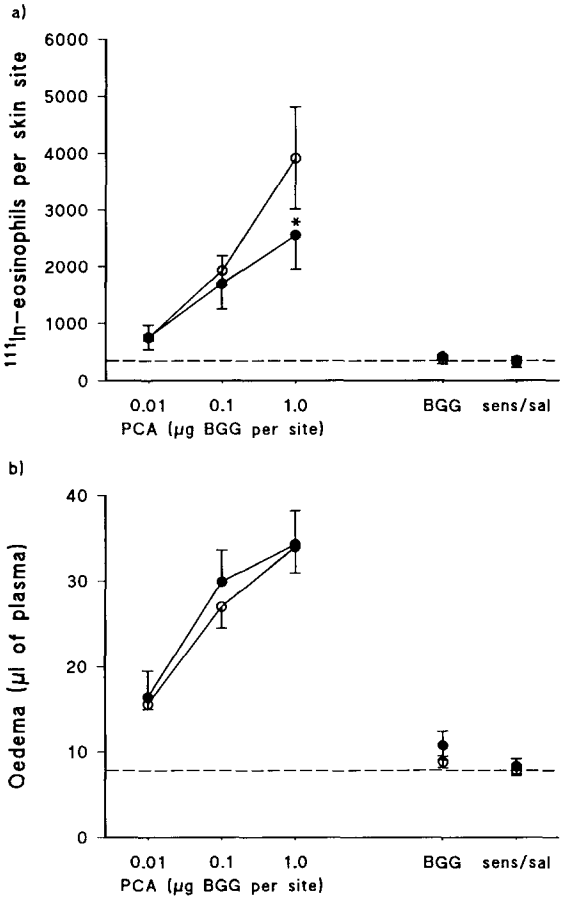


Fig. 4. Effect of systemic treatment with salbutamol (0.1 mg/kg, 3 h prior to i.v. injection of radiolabelled cells and albumin) on $^{111}$In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylactic (PCA) reaction. Control animals are shown by open circles and treated animals by filled circles. Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine γ-globulin) in saline-treated sites (shown as BGG) or sites previously sensitized with $IgG_1$-rich anti-sera (shown as PCA). The line across the graphs represents background values obtained in sensitized skin sites injected with saline (sens/sal). Results are means ± S.E.M. of four pairs of guinea pigs where * represents $P < 0.05$ when compared to control animals.

pretreatment (Table 3). Oedema formation in response to i.d. injection of zymosan-activated plasma (10% and 30% in saline) or histamine ($2.5 \times 10^{-8}$ mol per site) was inhibited when salmeterol was given as a 15 min, but not as a 3 h, pretreatment (Table 3). Responses to the lower dose of histamine ($2.5 \times 10^{-9}$ mol per site) were significantly inhibited by both pretreatments (Table 3). In contrast, oedema formation induced by zymosan-activated plasma 100% or PAF was unaltered by either treatment (Table 3).

## 4. Discussion

In this study, we investigated the effects of two $\beta_2$-adrenoceptor agonists on $^{111}$In-eosinophil accumulation and oedema formation in allergic and non-allergic inflammation in guinea pig skin. Locally administered salbutamol, a $\beta_2$-adrenoceptor agonist with a short duration of action, significantly reduced $^{111}$In-eosinophil accumulation in a passive cutaneous anaphylactic reaction and in response to i.d. injection of PAF. The inhibition by salbutamol of PAF-induced $^{111}$In-eosinophil accumulation was dose dependently reversed by DL-propranolol indicating that salbutamol was acting through β-adrenoceptors. This was further supported by the observation that D-propranolol, which shows little activity on β-adrenoceptors, was much weaker in reversing the inhibitory effects of salbutamol on PAF-induced $^{111}$In-eosinophil accumulation. Oedema formation in a passive cutaneous anaphylactic reaction was also inhibited by salbutamol. Similarly, a $\beta_2$-adrenoceptor agonist with a long duration of action, salmeterol, significantly reduced $^{111}$In-eosinophil accumulation in a passive cutaneous anaphylactic reaction and in response to PAF. The inhibitory effects of salmeterol were also reversed by DL-propranolol. Systemic injection of salbutamol 15 min, but not 3 h, prior

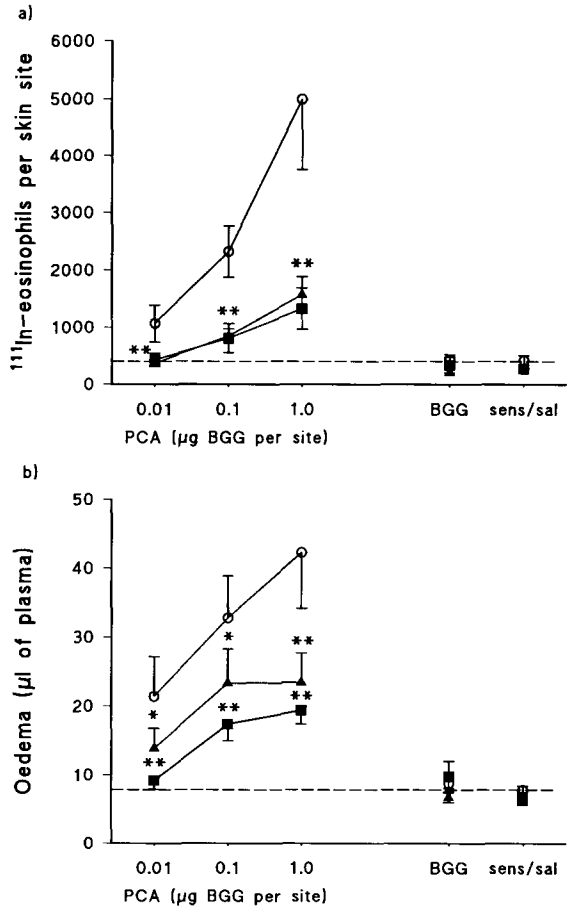


Fig. 5. Effect of systemic treatment with salmeterol (0.1 mg/kg, 15 min or 3 h prior to i.v. injection of radiolabelled cells and albumin) on $^{111}$In-eosinophil accumulation (a) and oedema formation (b) in a passive cutaneous anaphylactic reaction. Control animals are shown by open circles, 15 min pretreated animals by filled squares and 3 h pretreated animals by filled triangles. Inflammatory responses were assessed 2 h after i.d. injection of antigen (bovine γ-globulin) in saline-treated sites (shown as BGG) and sites previously sensitized with $IgG_1$-rich anti-sera (shown as PCA). The line across the graphs represents background values obtained in sensitized skin sites injected with saline (sens/sal) in control and salmeterol-treated animals. Results are means ± S.E.M. of five guinea pigs in each group where * and ** represent $P < 0.05$ and $P < 0.01$, respectively, when compared to control animals.

to i.v. injection of radiolabelled eosinophils and $^{125}$I-human serum albumin effectively inhibited $^{111}$In-eosinophil accumulation and oedema formation. Salmeterol effectively inhibited $^{111}$In-eosinophil accumulation and oedema formation in a passive cutaneous anaphylactic reaction and in response to other mediators when given 15 min or 3 h prior to i.v. injection of radiolabelled eosinophils and $^{125}$I-human serum albumin. Thus, both a short- and a long-acting $\beta_2$-adrenoceptor agonist had local and systemic anti-inflammatory effects in guinea pig skin but only systemic salmeterol was still effective when given 3 h prior to the experiment, reflecting its longer duration of action (Ball et al., 1991).

We previously showed that isoprenaline, a non-specific β-adrenoceptor agonist, significantly inhibited the accumulation of $^{111}$In-eosinophils in a passive cutaneous anaphylactic reaction and in response to i.d. injection of PAF and zymosan-activated plasma in guinea pig skin (Teixeira et al., 1993a). Similarly, prostaglandin $E_1$ inhibited $^{111}$In-eosinophil accumulation in a passive cutaneous anaphylactic reaction and in response to other inflammatory mediators (Teixeira et al., 1993a). These observations suggest that drugs which can elevate cyclic AMP within different cells possess significant acute anti-inflammatory effects in vivo. The inhibition of $^{111}$In-eosinophil accumulation induced by various inflammatory stimuli by rolipram, a type IV phosphodiesterase inhibitor, further supports a role for cyclic AMP elevation as a relevant anti-inflammatory approach (Teixeira et al., 1994b). Interestingly, neither isoprenaline, prostaglandins or rolipram inhibited the accumulation of $^{111}$In-neutrophils in guinea pig skin (Teixeira et al., 1993a, 1994b).

Recently, Whelan and Johnson (1992) and Whelan et al. (1993) reported on the anti-inflammatory effects of salbutamol, formoterol and salmeterol in guinea pig skin and lung. In the lung, all three compounds inhibited albumin accumulation at 30 min in response to inhaled histamine but salmeterol showed the longest duration of action. Both salmeterol and formoterol, but not salbutamol, inhibited the accumulation of eosinophils in the lung 24 h after aerosolized PAF. In the skin, local injection of all three drugs 30 min prior to the i.d. stimuli inhibited bradykinin-induced oedema formation after 30 min but only salmeterol inhibited zymosan-induced $^{111}$In-granulocyte accumulation after 4 h. They concluded that a long duration of action was necessary for $\beta_2$-adrenoceptor agonists to inhibit aspects of acute inflammation, such as granulocyte accumulation (Whelan and Johnson, 1992; Whelan et al., 1993). Continuous administration of salbutamol by an osmotic minipump implanted 5 days prior to an aerosol of PAF had no inhibitory effect on eosinophil accumulation in bronchoalveolar lavage fluid of guinea pigs performed 48 h later (Sanjar et al., 1990). Similarly, aerosolized salbutamol had no suppressive effect on eosinophil accumulation in guinea pig lung 72 h following antigen challenge of sensitized animals (Hutson et al., 1988). In the current experiments, both salbutamol and salmeterol inhibited $^{111}$In-eosinophil accumulation when given systemically or locally but systemic salmeterol was longer acting. Since in guinea pig skin most $^{111}$In-eosinophil accumulation occurs during the first hour after i.d. injection of stimuli (Weg et al., 1992), it is likely that salbutamol is still present at its site of action when administered locally or as a 15 min pretreatment, but not as a 3 h pretreatment. In contrast, and because of its longer duration of action, salmeterol is still active when given as a 3 h pretreatment. The

lack of effect of salbutamol on eosinophil accumulation in the studies by Whelan and Johnson (1992) and Hutson et al. (1988) could be related to the time course of eosinophil accumulation in guinea pig lung, possibly occurring after clearance of salbutamol. In the study by Sanjar et al. (1990), it is possible that the continuous administration of salbutamol may have induced tolerance to some of the effects of the drug as it has been demonstrated with another short-acting $\beta_2$-adrenoceptor agonist (O'Connor et al., 1992). These studies contrast with others which showed inhibition by the short-acting $\beta_2$-adrenoceptor agonist, fenoterol, of eosinophil accumulation in bronchoalveolar lavage fluid following antigen challenge of sensitized guinea pigs (Fugner, 1989). Similarly, systemic administration of isoprenaline suppressed sephadex-induced blood and lung eosinophilia in rats (Spicer et al., 1990). In addition, i.d. co-injection of terbutaline abrogated eosinophil accumulation on membrane filters applied to human skin 2.5 h after antigen challenge (Ting et al., 1983).

The observation that i.d. co-administration of salmeterol with inflammatory stimuli showed smaller threshold potency than salbutamol for inhibition of $^{111}$In-eosinophil accumulation contrasts with in vitro data suggesting that salmeterol is 8–10 times more potent than salbutamol on $\beta_2$-adrenoceptors (Ball et al., 1991; Butchers et al., 1991). Similarly, our data contrast with the greater potency of salmeterol compared to salbutamol at inhibiting bradykinin-induced oedema formation in guinea pig skin (Whelan et al., 1993). In our experiments, the $\beta$-adrenoceptor agonists were co-injected with the inflammatory stimuli and not given as a pretreatment as described by Whelan et al. (1993). Since salmeterol has a slower onset of action than salbutamol in vitro (Ball et al., 1991; Butchers et al., 1991), it is possible that some $^{111}$In-eosinophil accumulation has occurred by the time salmeterol starts acting in the skin. When injected 30 min prior to the i.d. injection of PAF, but not when co-injected, salmeterol inhibited PAF-induced $^{111}$In-eosinophil accumulation at $10^{-8}$ mol/site. At $10^{-9}$ mol/site, 30 min pretreatment with salmeterol inhibited PAF-induced $^{111}$In-eosinophil accumulation by 35% but this was not significant (data not shown). Together, these data indicate that salmeterol is more potent when given as a pretreatment rather than when co-injected with inflammatory stimuli.

The explanation for the inhibition of $^{111}$In-eosinophil but not $^{111}$In-neutrophil accumulation by isoprenaline (Teixeira et al., 1993a) and salbutamol (Whelan et al., 1993) is unknown, but it is possible that endothelial cells are a target for these drugs. Indeed, induction of the endothelial cell expression of vascular cell adhesion molecule-1 (VCAM-1), a ligand for VLA-4 present on eosinophils but not neutrophils, is inhibited by cyclic AMP-elevating agents (Pober et al., 1993). In contrast, endothelial cell expression of intercellular adhesion molecule-1 (ICAM-1, a ligand for CD11/CD18 expressed on both neutrophils and eosinophils) is not modulated by these agents (Pober et al., 1993).

$\beta_2$-Adrenoceptor agonists (both short- and long-acting) are well-known for their ability to inhibit mast cell degranulation in vitro (Butchers et al., 1991; Church and Hiroi, 1987). Thus, it is possible that at least part of the inhibitory effect of both salbutamol and salmeterol on radiolabelled eosinophil accumulation was mediated through inhibition of mast cell degranulation, especially in the passive cutaneous anaphylactic reaction. The observation that 3 h pretreatment with salbutamol partially inhibited $^{111}$In-eosinophil accumulation in a passive cutaneous anaphylactic reaction (1 $\mu$g bovine $\gamma$-globulin per site, Fig. 4), but not $^{111}$In-eosinophil accumulation induced by other mediators (Table 3), provides support for this proposal. Furthermore, 3 h pretreatment with salmeterol significantly inhibited oedema formation in the passive cutaneous anaphylactic reaction, but, generally, it had less effect against the mediators tested (Fig. 5, Table 3). However, there is no evidence for a mast cell component in histamine- or PAF-induced $^{111}$In-eosinophil accumulation, but these responses were effectively inhibited by both salbutamol and salmeterol suggesting a site of action other than the mast cell for these drugs.

We conclude that a long duration of action of $\beta_2$-adrenoceptor agonists is not necessary to demonstrate acute anti-inflammatory effects on eosinophil accumulation in guinea pig skin.

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