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Salmeterol inhibits anaphylactic histamine release from guinea-pig isolated mast cells

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Abstract—Salmeterol (1 nM–100 μM) showed an inhibitory action on anaphylactic histamine release from mast cells, isolated from pleural and peritoneal cavities of actively sensitized guinea-pigs and stimulated by incubation with allergen. The effect is concentration-dependent and is reduced by the β-adrenoceptor antagonist propranolol (1 μM). This study supports the hypothesis of an anti-inflammatory property of salmeterol, which concerns cells involved in the early phases of asthma.

The inflammatory component in the pathogenesis of bronchial asthma draws attention to drugs that act by promoting inhibitory mechanisms on both bronchomotor tone and activation of cells implicated in inflammatory processes and liberation of mediators. The presence of neurotransmitter receptors on mast cell membrane is important for modulating the release of inflammatory mediators, both pre-formed and newly-formed in response to appropriate stimuli.

Salmeterol, a long-acting β_2-adrenergic receptor agonist (Bradshaw et al 1987), has been introduced into the therapy of asthma as a bronchodilator. Other mechanisms have been suggested, as it also appears to possess anti-inflammatory properties. Salmeterol has been shown to inhibit the release of mediators from fragments of human lung during in-vitro anaphylactic reactions (Butchers et al 1991) and from stimulated human alveolar macrophages (Baker & Fuller 1990). Salmeterol given by inhalation reduced the activity of eosinophils in bronchoalveolar lavage of asthmatics (Dahl et al 1991). In animal models salmeterol has been found to inhibit increased permeability to plasma proteins in the guinea-pig pulmonary microcirculation caused by histamine, and to prevent eosinophil accumulation in the lung after platelet-activating factor or allergen administration to guinea-pigs (Johnson 1991). On the basis of in-vivo observations in man, other authors do not agree with the likelihood of anti-inflammatory properties of salmeterol (O'Shaughnessy et al 1991; Taylor et al 1992).

Our previous observations have shown that salmeterol is effective in protecting actively sensitized guinea-pigs from bronchospasm evoked by an allergenic aerosol, and to a lesser extent from histamine aerosol (Zilletti et al 1992). The drug also inhibits the release of spasmogenic substances (histamine, SRS-A) from segments of guinea-pig trachea during the course of anaphylactic reactions in-vitro (Zilletti et al 1992).

The experiments reported here have been conducted with the object of establishing whether salmeterol has a direct action on the release of histamine from mast cells isolated from the serous pleura and peritoneum of sensitized guinea-pigs.

Materials and methods

Drugs. The following were used: histamine dihydrochloride (Carlo Erba), isoprenaline sulphate, (+)-propranolol, salmeterol xinofoate (kindly provided by Glaxo SpA), collagenase type I (Sigma), BSA fraction V FFA (Boerhinger Mannheim), *o*-phthalaldehyde (Sigma). All reagents used were of highest grade (Merck).

Sensitization of the animals. Male albino guinea-pigs, 250–300 g, were sensitized with a suspension of ovalbumin and Freund's incomplete adjuvant, administered subcutaneously. They were used after 6–8 weeks (Dale 1965).

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Collection and isolation of mast cells. The method of Pearce & Ennis (1980) was followed, with modification. The guinea-pigs were killed by a blow on the neck and exsanguinated. For isolation of mast cells a solution at pH 6·00 (NaCl 154 mM, KH_2PO_4 6 mM) with a 1 mg mL^{-1} collagenase was injected into the serous cavities, 15 mL into the peritoneal and 4 mL into the pleural cavities. After light massage for 15 min the liquid was collected from the peritoneal cavity opened with an incision along the median line of the abdomen and from the pleural cavities opened from the diaphragm immediately above the xiphoid process. The cells, always maintained in ice, were washed in a solution at pH 7·4 (containing (mM): NaCl 145, KCl 2·5, $CaCl_2$ 0·9, Na_2HPO_4 1·3, glucose 5, bovine serum albumin 1 mg mL^{-1}), then suspended in 1 mL of the same solution. The vitality of the cells was checked by exclusion of 0·4% trypan blue. The mast cell count was determined previous to colouration with 0·1% toluidine blue. Mast cells represented 80–85% of the cells removed from the peritoneal and pleural folds.

Anaphylactic reaction and fluorimetric assay of histamine. After dilution and subdivision to 130 000–150 000 cells/sample, the cellular suspension was incubated at 37°C in the presence or the absence (controls) of drug for 20–40 min and with ovalbumin for a further 30 min. After centrifugation (800 *g*, 15 min) the supernatant was separated and 2 mL 0·1 M HCl added to the pellet and vigorously agitated to extract histamine.

Histamine content in the supernatant and in the hydrochloric acid solution obtained from the pellet was determined by the fluorimetric method of Shore et al (1959) modified by Kremzer & Wilson (1961) to be 1·5–5 μg/10^6 cells.

The release of histamine in the supernatant was expressed as a percentage of total histamine content in the supernatant and in the pellet. The spontaneous release of histamine was less than 8% and was subtracted in each sample.

Results

The anaphylactic reaction evoked by 1, 5 and 10 mg mL^{-1} ovalbumin caused an average release of 34·4, 68·8 and 70·8% of the total histamine content, respectively.

Salmeterol (1 nM–100 μM) inhibited histamine release from isolated mast cells at each concentration of ovalbumin used. The effect of salmeterol is concentration-related (Fig. 1), with an IC50 = 95 nM when 5 mg mL^{-1} ovalbumin was used. The efficacy was slightly less with 10 mg mL^{-1} ovalbumin, while at 1 mg mL^{-1} the calculated IC50 was 3·55 nM.

In comparative tests, 1 nM–10 μM isoprenaline inhibited the histamine release in a dose-related manner, being about 16-fold more potent than salmeterol. Propranolol (1 μM) was able to antagonize the inhibitory effect of salmeterol (Table 1), although not completely. It is likely that the inhibitory actions of both salmeterol and isoprenaline are mediated via β-adrenergic receptors. However, the incomplete antagonism of propranolol against the inhibitory effect of salmeterol suggests that a small part of the efficiency of the drug under study can be related to other properties of the molecule.

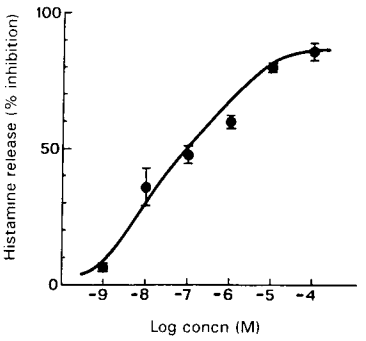

FIG. 1. Concentration-dependent inhibitory effect of salmeterol on anaphylactic histamine release from guinea-pig serosal mast cells. Values are expressed as mean ± s.e.m.

Table 1. Partial antagonism by 1 μM propranolol of the inhibitory effect of salmeterol. Ovalbumin (5 mg mL^{-1}) was used to induce anaphylaxis.

	Histamine release (%)	
Treatment	Salmeterol	Salmeterol with propranolol
Control	80·5 ± 5·3	
Salmeterol		
10^{-8} M	60·8 ± 5·4	65·2 ± 1·0
10^{-7} M	54·4 ± 4·9	63 ± 1·0
10^{-6} M	35·4 ± 3·2	62 ± 0·3
10^{-5} M	28·2 ± 4·0	63·3 ± 0·4

Values are mean ± s.e.

Discussion

Guinea-pig isolated mast cells constitute a suitable experimental model for studying the influence of drugs on the liberation of mediators of allergic inflammation. This method allows one to evaluate the role played on mast cells by drugs which have been shown to be active in guinea-pigs, both in in-vivo models against allergen-induced bronchospasm, and in-vitro in isolated organs.

Salmeterol is active in protecting actively sensitized guinea-pigs against the microshock provoked by ovalbumin aerosol and is able to reduce the release of mediators (Zilletti et al 1992) as well as to inhibit contractions (unpublished data) in isolated tracheal segments during challenge with antigen. In the present study, salmeterol inhibits histamine release, in a dose-related manner, from mast cells isolated from the sensitized guinea-pig. This effect appears to be mostly related to activation of β_2-adrenergic receptors, since it is antagonized by propranolol. Thus, the protective effect of salmeterol against allergic bronchospasm in guinea-pigs may be, in part, related to an anti-inflammatory property, as seems to occur in man.

In man, an anti-inflammatory property of salmeterol is supported by in-vitro studies, using isolated tissues. Salmeterol inhibits both the release of histamine and eicosanoids immunologically evoked from human lung (Butchers et al 1991) and the contractions evoked by anti-IgE in bronchial strips (Gorenne et al 1992). Therefore, on the basis of mediators released and of the stimuli used, mast cells or basophils appear to be targets of salmeterol.

The potency of salmeterol on guinea-pig isolated mast cells would be about one-sixteenth that of isoprenaline, as can be determined from the ratios of the IC50 values. It is to be recalled that isoprenaline not only acts on β_2-adrenoceptors but is also a potent agonist of β_1-receptors. From the therapeutic point of view, β_2-adrenergic agonists are pre-eminent in the treatment of the asthmatic syndrome, since they do not present undesirable effects due to activation of β_1-receptors. Among the specific β_2-agonists the most closely chemically related, salbutamol, has been found less potent than salmeterol in in-vitro experiments (Butchers et al 1991).

In conclusion, our study indicates that salmeterol may protect the sensitized individual partly by affecting mast cell activity. As suggested by other reports (Butchers et al 1991; Gorenne et al 1992), the drug effect on mast cells could be long-lasting. Mast cells are recognized to play an important role in the early phase of bronchospastic response to allergen and administration of salmeterol could prevent an eventual anaphylactic reaction in the airways. Salmeterol has been shown to improve both bronchial hyper-responsiveness and symptoms during the late phase of allergic asthma (Twentyman et al 1990); these effects have been ascribed to a prolonged bronchodilator action, whereas an influence on inflammatory cell or mediators of these conditions has yet to be demonstrated. Mast cells can contribute to the development of the late phase of bronchial hyper-reactivity, probably through the production of substances different from the classical mediators, in particular, cytokines.

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References

Baker, A. J., Fuller, R. W. (1990) Anti-inflammatory effect of salmeterol on human alveolar macrophages. Am. Rev. Respir. Dis. 141 (Suppl.): A394

Bradshaw, J., Brittain, R. T., Coleman, R. A., Jack, D., Kennedy, I., Lunts, L. H. C., Skidmore, I. F. (1987) The design of salmeterol, a long acting, selective β_2-adrenoceptor agonist. Br. J. Pharmacol. 92: 590P

Butchers, P. R., Vardey, C. J., Johnson, M. (1991) Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br. J. Pharmacol. 104: 672–676

Dahl, R., Pedersen, B., Venge, P. (1991) The influence of inhaled salmeterol on bronchial inflammation: a bronchoalveolar lavage study in patients with bronchial asthma. Am. Rev. Respir. Dis. 143 (Suppl.): A649

Dale, M. M. (1965) The applicability of anaphylactic tests in studies of antigen mixtures. Immunology 8: 435–443

Gorenne, I., Labat, C., Norel, X., Guillet, C., Cavero, I., Brink, C. (1992) Anti-inflammatory activity of beta$_2$-agonists in human airways (HB). Pharmacol. Res. 26 (Suppl. 1): 33

Johnson, M. (1991) Salmeterol: a novel drug for the treatment of asthma. Agents Actions 34 (Suppl.): 79–95

Kremzer, L. T., Wilson, I. B. (1961) A procedure for determination of histamine. Biochem. Biophys. Acta 50: 364

O'Shaughnessy, K. M., Taylor, I. K., Fuller, R. W. (1991) Letter to the Editor. Lancet 337: 45–46

Pearce, F. L., Ennis, M. (1980) Isolation and some properties of mast cells from the mesentery of the rat and guinea pig. Agents Actions 10: 124–131

Shore, P. A., Burkhalter, A., Cohn, V. R. (1959) A method for the fluorimetric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127: 182–186

Taylor, I. K., O'Shaughnessy, K. M., Choudry, N. B., Adachi, M., Palmer, J. B. D., Fuller, R. W. (1992) A comparative study in atopic subjects with asthma of the effects of salmeterol and salbutamol on allergen-induced bronchoconstriction, increase in airway reactivity, and increase in urinary leukotriene E_4 excretion. J. Allergy Clin. Immunol. 89: 575–583

Twentyman, O. P., Finnerty, J. P., Harris, A., Palmer, J., Holgate, S. T. (1990) Protection against allergen-induced asthma by salmeterol. Lancet 336: 1338–1342

Zilletti, L., Franchi-Micheli, S., Gentilini, G. (1992) Inhibitory mechanisms and modulation of anaphylactic reaction in guinea-pig airways. Pharmacol. Res. 26 (Suppl. 2): 38–39