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Effects of sodium cromoglycate analogues on the immunological release of slow reacting substance of anaphylaxis and histamine from human and guinea-pig lung in vitro

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Histamine and slow-reacting substance of anaphylaxis (SRS-A) are released during antigen challenge of asthmatic human lung tissue in vitro (Brocklehurst 1960). The contractile effects of histamine and SRS-A on human isolated bronchial smooth muscle preparations have suggested a role for these mediators in the pathogenesis of allergen-provoked asthma (Sheard & Blair 1970). Furthermore, drugs which inhibit the antigen-induced release of histamine and SRS-A from human lung tissue in vitro such as β-adrenoceptor agonists (Orange et al 1971) and disodium cromoglycate (Sheard & Blair 1970) have been useful in the treatment of asthma.

In the search for new anti-asthmatic drugs a variety of animal models has been used but the poor correlation between activity in these models and clinical efficacy has led to the wider use of human lung as a potentially more relevant tissue for evaluation of drug efficacy (Church & Gradidge 1980). However, the ability of compounds to inhibit histamine release from passively sensitized human lung tissue in vitro was not related to clinical efficacy in asthma (Church & Gradidge 1980).

Leukotriene D which has recently been identified as a major component of SRS-A (Morris et al 1980) is more potent than histamine in contracting human bronchial smooth muscle strips (Hanna et al 1981). Thus, it may be more relevant to determine the inhibitory effects of drugs on SRS-A release rather than on histamine release as a possible correlate of clinical efficacy. This paper reports the effects of some analogues of disodium cromoglycate (DSCG) on the release of SRS-A and histamine from human and guinea-pig lung tissue. The DSCG analogues used were FPL57787 (6,7,8,9-tetrahydro-5-hydroxy-4-oxo-10-propyl-4H-naphtho(2,3-b)pyran-2 carboxylic acid) (Augstein et al 1977); FPL52791 (6,8-di-t-butyl-4-oxo-4H-1-benzopyran-2-carboxylic acid) (Augstein et al 1976) and FPL58668 (4,6-dioxo-10-propyl-4H,6H-benzo(1,2-b: 5,4-b) dipyran-2,8-dicarboxylic acid) (Oxhøj & Hyldebrandt 1980). These compounds were tested as the soluble sodium (57787 and 52791) or disodium (58668) salts.

Method

Macroscopically normal lung tissue which was obtained from patients undergoing thoracotomy for carcinoma of the lung was washed free of blood with Tyrode solution and cut into fragments approximately 3 mm^3. The fragments were incubated at room temperature overnight in serum from an atopic subject (specific RAST titre against *Dermatophagoides pteronyssinus* 4+). For experiments with guinea-pig lung, chopped tissue was prepared from animals sensitized by intraperitoneal and subcutaneous injection of ovalbumin (100 mg) 3–5 weeks previously. Lung fragments from either species were divided into replicates (250 mg) and suspended in Tyrode solution with or without drug (concentration range 10^{-7}–10^{-4} mol $litre^{-1}$) for 15 min at 37°C before addition of antigen (*D. pteronyssinus* extract 200 Noon units ml^{-1} for human lung; ovalbumin 50 μg ml^{-1} for guinea-pig lung). After a further 15 min at 37°, incubation pots were placed in an ice bath. The content of SRS-A in the supernatant was determined by bioassay and compared with a partially purified laboratory standard (see Engineer et al 1978). Samples of supernatant were stored in 0·4 mol $litre^{-1}$ $HClO_4$ at −20° for subsequent fluorimetric assay of histamine (Engineer et al 1978). Residual histamine was released from the lung tissue by boiling and the histamine release during antigen challenge was expressed as a percentage of the original tissue histamine and corrected for spontaneous release.

Results and discussion

In experiments with human lung tissue FPL57787 produced a dose-dependent inhibition of SRS-A release (Fig. 1). Whereas the release of SRS-A was significantly reduced by concentrations of FPL57787 in the range 10^{-6}–10^{-4} mol $litre^{-1}$, significant inhibition of histamine release was evident with only the highest concentration of

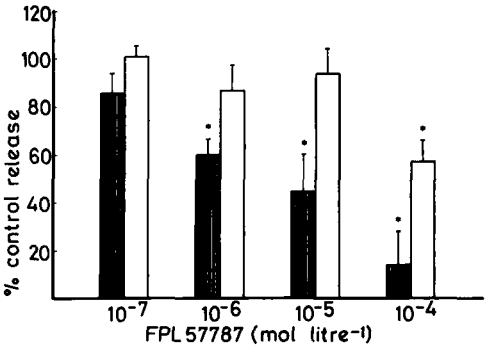

FIG 1. Effect of FPL57787 on antigen-induced release of SRS-A (lined columns) and histamine (open columns) from human lung in vitro. Mean values were obtained with tissue from 3 to 5 individuals and vertical lines denote s.e. * $P < 0.05$ (*t*-test).

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Table 1. Effects of DSCG analogues on antigen-induced release of SRS-A and histamine from human lung (expressed as percent of control values). Each value is the mean ± s.e.m. of 3–5 experiments.

	FPL58668 (mol litre^{-1})			FPL52791 (mol litre^{-1})		
	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}
SRS-A	61·5 ± 13·0	62·5 ± 6·9†	59·4 ± 9·1†	109·9 ± 23·6	100·3 ± 16·9	78·6 ± 7·0
Histamine	99·6 ± 9·9	93·7 ± 13·4	95·3 ± 10·7	107·6 ± 13·8	108·8 ± 14·1	100·0 ± 8·7

† $P < 0.05$, t-test.

FPL57787 (10^{-4} mol litre^{-1}). FPL58668 caused an inhibition of SRS-A which was not dose-related, since mean values for SRS-A release in the presence of 10^{-7}, 10^{-6} and 10^{-5} mol litre^{-1} of FPL58668 were all between 59 and 62% of control values and not significantly different from each other (Table 1). Histamine release was unaffected at these concentrations. FPL52791 had no significant effect on SRS-A or histamine release at concentrations of 10^{-7}–10^{-5} mol litre^{-1} (Table 1). The antigen-induced release of histamine from human lung varied between tissue samples from different individuals (range 9·2–57·5% of original tissue content; mean ± s.e. 27·8 ± 4·2%, n = 12), as found by Sheard & Blair (1970), and Church & Gradidge (1980).

In three experiments with guinea-pig lung, FPL57787 produced a small but significant inhibition of SRS-A release in a concentration of 3×10^{-5} mol litre^{-1} (mean ± s.e. 73·5 ± 4·7% of control; $P < 0.05$) but lower concentrations were ineffective. At a concentration of 3×10^{-5} mol litre^{-1} FPL57787 did not inhibit histamine release (mean ± s.e. 92·9 ± 7·1% of control) but at 10^{-4} mol litre^{-1} histamine release was reduced to 61·4 ± 8·5% of control. Neither FPL52791 nor FPL58668 (concentration range 10^{-7}–10^{-4} mol litre^{-1}) significantly affected the release of SRS-A or histamine.

The present experiments have shown that FPL57787, which was found to be effective in a single-blind clinical trial in perennial extrinsic asthma (Dahl 1980), inhibited SRS-A release and in higher concentrations also histamine. The concentrations of FPL57787 which inhibited histamine release were higher than those reported by Church & Gradidge (1980). This difference may have been due to the marked variability in responses of human lung tissue in vitro evident in the two studies.

Although FPL58668 produced a significant reduction in the release of SRS-A this inhibition was not dose-related. Since a similar pattern of inhibition has also been observed with DSCG (see Butchers et al 1979) this finding should not preclude FPL58668 from clinical evaluation.

These studies have suggested that inhibitory effects of drugs on the immunological release of SRS-A from human lung tissue in vitro may be a more reliable determinant of clinical efficacy than its inhibitory effects on the release of histamine. However, factors other than inhibition of mediator release may contribute to the clinical efficacy of these compounds, as has been suggested for DSCG (Harries et al 1981).

These studies also illustrate the different responses of guinea-pig and human lung tissue to analogues of DSCG. Using the sensitization procedures described, the antigen-antibody response involved different immunoglobins, IgE in human lung and IgG in guinea-pig lung, which may account for the different responses to DSCG and its analogues.

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