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The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine

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1 Three drugs have been tested for activity against antigen-induced histamine release from passively sensitized human lung fragments after increasing periods of pre-incubation before challenge.

2 After 30 s pre-incubation, sodium cromoglycate inhibited histamine release in the concentration range 0.2–200 μM, producing a maximum inhibition of 33.0%. As the pretreatment period was extended, tolerance developed in a dose-related manner, resulting in a 48.3% and 82.8% loss of activity of the 200 μM dose after 60 min and 19 h pre-incubation, respectively. Tolerance was independent of extracellular calcium and was poorly reversible. Lung tissue desensitized to cromoglycate was cross-tolerant to the related drug, bufrolin, but not to salbutamol or chlorpromazine.

3 In acute studies, salbutamol (0.03–3.0 μM) produced dose-related inhibition of histamine release, with a maximum inhibition of 72.2%. The effect was blocked stereoselectively by 1 μM propranolol, suggesting that it occurred through an interaction with lung β-adrenoceptors. Increasing the pre-incubation time with salbutamol from 30 s to 19 h did not produce tolerance. Inhibition produced by incubation with salbutamol for 19 h was totally prevented when propranolol was added at the beginning of the pre-incubation period, indicating that it resulted from stimulation of β-receptors and not from a non-specific or toxic effect. However, studies of reversibility of effect through washing or late addition of propranolol did indicate some change in the nature of salbutamol inhibition with time.

4 Chlorpromazine was a weak inhibitor of immunological histamine release. A 100 μM concentration was ineffective after 30 s pre-incubation but its activity increased with time. Pre-incubation of lung fragments with this concentration for 1 h or longer, or with a 1 mM dose for a shorter period, provoked histamine release in the absence of antigen. Effects of chlorpromazine were not reversed by washing.

5 The different characteristics shown by sodium cromoglycate, salbutamol and chlorpromazine indicate that these drugs inhibit histamine release by interfering with the secretory mechanisms in different ways.

Introduction

Most prospective drugs for the treatment of human allergic disease are initially assessed in animal models of anaphylaxis, predominantly those based on the rat mast cell. However, since there are differences in the ultrastructure and histamine-releasing capacities of human and rat mast cells (Lichtenstein, Foreman, Conroy, Marone & Newball, 1979; Caulfield, Lewis, Hein & Austen, 1980), it is possible that responses of the cells to drugs also differ.

In rat mast cells, sodium cromoglycate is a consistent and reasonably effective inhibitor of histamine release induced by IgE-antibody interaction or by artificial stimuli (Orr, 1977). In passively sensitized human lung fragments challenged with specific antigen, this drug has only a partial and variable inhibitory effect (Sheard & Blair, 1970; Morr, 1978; Sharpe, Ross & Spicer, 1978; Butchers, Fullarton, Skidmore, Thomson, Vardey & Wheeldon, 1979;

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Church & Gradidge, 1980b; Young & Church, 1982). With β-adrenoceptor stimulants, the situation is reversed. Although capable of interacting with β-receptors on the surface of rat mast cells (Donlon, Hunt, Catravas & Kaliner, 1980), the β-stimulant isoprenaline is inconsistent in its ability to increase the cellular levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP; Ishizaka, 1981) and has negligible inhibitory effect on mediator release (Johnson & Moran, 1970). However, in human lung fragments, this and other β-stimulants are potent inhibitors of release (Assem & Schild, 1969; Butchers *et al.*, 1979; Young & Church, 1983). Histamine H_1-receptor antagonists produce similar effects on both tissues; at relatively low concentrations, they inhibit mediator release by a mechanism unrelated to their H_1-receptor-blocking property whilst at higher concentrations they actually induce release (Mota & Dias da Silva, 1960; Frisk-Holmberg, 1972; Church & Gradidge, 1980a).

A characteristic feature of sodium cromoglycate activity in rat mast cells is the rapid development of tolerance, an effect which cannot be explained by a short duration of action or drug metabolism (Kusner, Dubnick & Herzig, 1973; Thomson & Evans, 1973). In addition to becoming tolerant to the effects of sodium cromoglycate, mast cells pretreated with this drug become cross-tolerant to related compounds, a feature used to define cromoglycate-like activity (Marshall, Thomson & Evans, 1976; Herzig, Kusner, Fox & Kaplan, 1977).

In preliminary investigations, we showed that tolerance to cromoglycate may also develop in human lung fragments, an effect not seen with either the β-adrenoceptor stimulant, salbutamol, or the H_1-receptor antagonist, chlorpromazine (Young & Church, 1980). Here, we extend these observations to characterize the inhibitory activities of all three agents against antigen-induced histamine release from human lung.

Methods

Preparation, sensitization and challenge of human lung fragments

Human lung obtained within 1 h of resection was chopped finely with scissors, passively sensitized overnight with serum pooled from 2 grass pollen-sensitive donors, challenged by 15 min incubation with Timothy pollen antigen (10 μg/ml) and histamine release measured spectrofluorimetrically, as described previously (Young & Church, 1983). Antigen-induced release was routinely calculated from six 200 mg replicates and was corrected for spontaneous release of histamine by subtracting the amount liberated from lung fragments incubated in the absence of antigen. In each experiment, drug activity was expressed as the mean percentage inhibition of histamine release in three or four lung replicates.

Time-response experiments

In experiments where lung fragments were incubated with drugs for different times before antigen challenge, results were compared with replicates incubated for a similar time in the absence of drug. With 19 h pre-incubation, replicates were thoroughly washed in PBS containing drug and the drug renewed 15 min before challenge. Controls were treated similarly in the absence of drug. All pre-incubations of 1 h or less were performed at 37°C. In 19 h experiments, tissues were incubated with drug for 18 h at room temperature followed by 1 h at 37°C.

Experiments comparing development of cromoglycate tolerance in the presence and absence of calcium.

Lung fragments were incubated with sodium cromoglycate, 200 μM, at 37°C for 1 h in either complete PBS or in calcium-free PBS containing 0.26 mM EGTA. Five minutes before challenge, all replicates were washed with, and resuspended in, the appropriate buffer containing drug (EGTA was omitted from Ca-free solution). Calcium-deprived samples were restored to the normal calcium concentration 5 s before antigen challenge, by adding 0.1 ml of a 20 mM calcium chloride solution to the 2 ml incubation medium. Results were compared with those from replicates treated in a similar manner, except that cromoglycate was only added 30 s before challenge.

Experiments on reversibility of drug effects by washing

To determine whether the acute effects of drugs could be reversed by washing, sensitized lung fragments were incubated, in bulk, with drug for 5 min at 37°C. One half of the fragments was then divided into 200 mg replicates and challenged immediately with antigen. The remainder was washed by stirring with 250 ml of PBS for 2 min, divided into 200 mg replicates and challenged. Wash-out experiments after 19 h incubation with drug were performed in an identical manner except that the washing was performed 15 min before challenge.

Cross-tolerance experiments

Replicates of human lung fragments were incubated with sodium cromoglycate for 18 h at room temperature followed by 1 h at 37°C. They were then washed thoroughly with PBS and incubated further for 30 s

with sodium cromoglycate (200 μM) or bufrolin (ICI 74917, 10 μM), or for 5 min with salbutamol (0.1 μM) or chlorpromazine (100 μM), before challenge. The effects of the drugs under these circumstances were compared with their effects in replicates incubated for 19 h without cromoglycate.

Statistics

The statistical significance of differences between means was assessed by the paired Student's *t* test or analysis of variance. Differences were only considered significant when $P < 0.05$.

Drugs

Sodium cromoglycate was kindly donated by Fisons Pharmaceuticals Ltd.; salbutamol sulphate by Glaxo Group Research; chlorpromazine hydrochloride by May and Baker Ltd.; (±)-propranolol, (+)-propranolol and bufrolin (ICI 74917, 6-*n*-butyl-2,8-dicarboxy-4,10-dioxo-1,4,7,10-tetrahydro-1,7-phenanthroline, disodium salt) by ICI Pharmaceuticals. EGTA (ethyleneglycol-*bis*-(β-aminoethyl ether) N,N[1]-tetraacetic acid) was purchased from Sigma. Drugs were always prepared afresh in phosphate buffered saline (PBS), at pH 7.2 (PBS mM: NaCl 137, KCl 2.7, $MgCl_2$ 0.5, $CaCl_2$ 1.0, Na_2HPO_4 8, KH_2PO_4 1.5 and glucose 5.5).

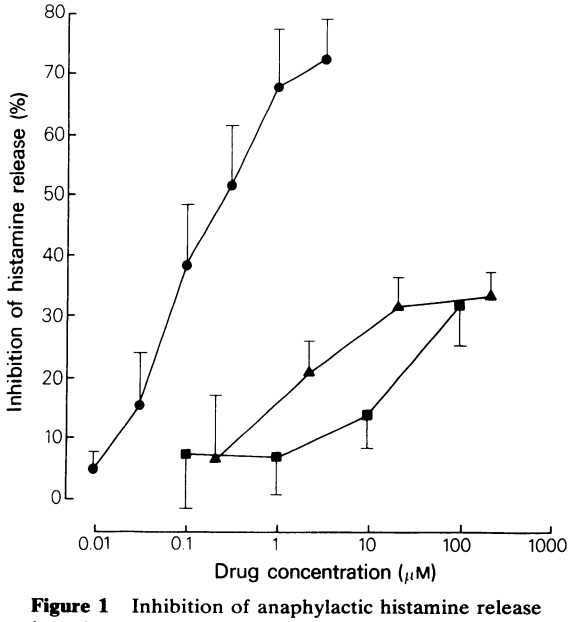

Figure 1 Inhibition of anaphylactic histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. Sodium cromoglycate (▲, 9 experiments) and salbutamol (●, 5 experiments) were added 30 s and chlorpromazine (■, 5 experiments) was added 5 min before antigen. Each result is expressed as mean with vertical lines indicating s.e.mean. Antigen-induced histamine release in these experiments was 20.1 ± 2.3% (Range = 6.0–35.5%).

Results

Concentration-related effects of sodium cromoglycate, salbutamol and chlorpromazine

The relationship between drug concentration and inhibition of histamine release was established by pre-incubating lung fragments with drug for 30 s (cromoglycate, salbutamol) or 5 min (chlorpromazine) before challenge (Figure 1). Sodium cromoglycate produced significant inhibition at concentrations of 2, 20 and 200 μM, the two highest doses examined showing a similar effect (31.4 ± 4.7% and 33.0 ± 4.2%). Salbutamol gave a significant, concentration-related inhibition over the range 0.03–3 μM, the maximum response being 72.2 ± 6.5%. With chlorpromazine, a significant inhibitory effect was seen only at 100 μM (31.3 ± 6.2% inhibition). This concentration slightly increased the spontaneous release of histamine and, when it was raised to 1 mM in three experiments, spontaneous release increased markedly from 2.2 ± 0.5% to 48.7 ± 5.3% of the total histamine in the tissue.

Characteristics of sodium cromoglycate inhibition

The time-dependency of sodium cromoglycate inhibition was examined in 9 experiments in which lung fragments were incubated with concentrations of 2, 20 or 200 μM for between 30 s and 19 h before challenge (Figure 2a). At 2 μM, activity developed slowly, significant inhibition being reached only after 15 min pre-incubation (22.4 ± 5.7%, $P < 0.05$), after which inhibition declined. At 20 and 200 μM, sodium cromoglycate was maximally effective when pre-incubated with lung fragments for 30 s before challenge, producing 29.8 ± 1.9% and 30.0 ± 1.9% inhibition, respectively. Prolongation of the pre-incubation period led to a gradual loss of activity; with 1 h pre-incubation, the activity of 20 and 200 μM sodium cromoglycate was 55.7% and 48.3%, respectively, less than that observed with 30 s pre-incubation ($P < 0.05$ in both cases). Further extension of the pre-incubation time to 19 h resulted in an 82.8% loss of activity of the 200 μM dose (30.0% → 6.3% inhibition).

The influence of extracellular calcium on the development of cromoglycate tolerance was examined in two experiments. Pre-incubation with 200 μM sodium cromoglycate for 30 s in the presence or absence of calcium produced 38.9% and 33.8% in-

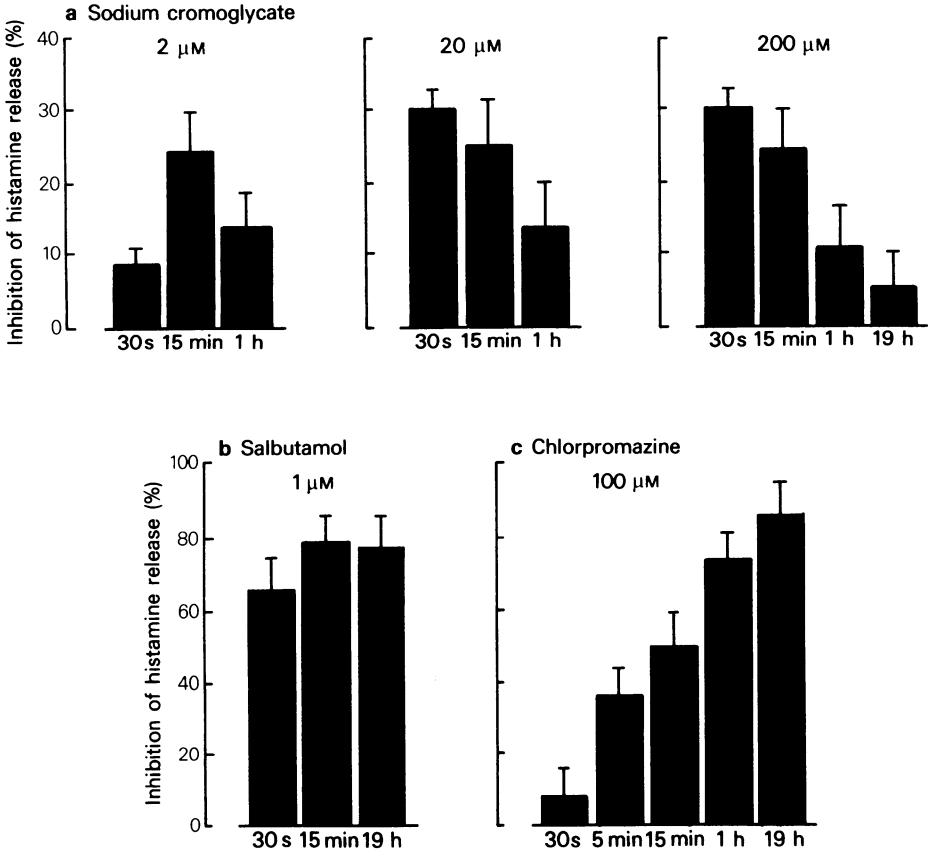

Figure 2 Time-dependency of the activity of sodium cromoglycate, salbutamol and chlorpromazine in human lung fragments. Each result is the mean (vertical lines indicate s.e.mean) of the inhibition of histamine release produced by drugs added at the stated time before challenge, as follows: (a) sodium cromoglycate, 2, 20 and 200 μM, each in 9 experiments; (b) salbutamol, 1 μM, in 6 experiments; (c) chlorpromazine, 100 μM, in 4–6 experiments. Antigen-induced histamine release in this series was 14.1 ± 2.0% (Range 4.9–35.5%). In all 19 h pre-incubation studies, tissues were washed and the drug renewed 15 or 5 min before challenge.

hibition of histamine release, respectively. Following a 1 h pre-incubation, the corresponding inhibitions were 12.4% and 9.6%, respectively, i.e. tolerance developed to a similar extent in both groups.

To test the reversibility of drug effect by washing, lung fragments were incubated with sodium cromoglycate for 5 min or 19 h, washed thoroughly and challenged with antigen. The results (Table 1) show that inhibition of histamine release produced by 5 min pre-incubation was completely removed by washing, an effect which was obscured in the 19 h samples by drug tolerance.

Characteristics of salbutamol inhibition

In contrast to sodium cromoglycate, the inhibitory effect of salbutamol on histamine release from human lung fragments was largely independent of the pre-incubation period (Figure 2b). At 30 s, salbutamol, 1 μM, inhibited release by 64.7 ± 8.4% whereas pre-incubation for 15 min and 19 h produced 78.3 ± 6.6% and 78.1 ± 6.6% inhibition of release, respectively.

Following 5 min pre-incubation with salbutamol, 1 μM, washing readily reversed the inhibition. However, the inhibition produced by 19 h exposure to the drug was not reversed by washing (Table 1).

The β-adrenoceptor antagonist, (±)-propranolol, was used to investigate the dependence of salbutamol activity on an interaction with β-adrenoceptors. In five experiments, the inhibition produced by 5 min pre-incubation with salbutamol, 0.1 μM, was reduced from 33.9 ± 9.1% to 3.2 ± 4.6% by addition of propranolol, 1 μM, 2.5 min before the β-stimulant.

Table 1 Reversibility by washing of the inhibitory effects of sodium cromoglycate, salbutamol and chlorpromazine in human lung fragments

Drug (concentration)	*Time of pre-incubation*	*Inhibition of histamine release (%) No wash*	*With wash*	n
Sodium cromoglycate (200 μM)	5 min	61.0 ± 3.8	2.1 ± 7.3	1
	19 h	6.3 ± 4.9	6.9 ± 6.2	6
Salbutamol (1 μM)	5 min	61.4 ± 5.8	9.4 ± 12.4	2
	19 h	78.1 ± 6.6	61.5 ± 6.9	6
Chlorpromazine (100 μM)	5 min	34.4 ± 7.0	39.6 ± 3.8	2
	19 h	75.4 ± 6.0	86.4 ± 6.8	4

Lung fragments were incubated with drugs for 5 min or 19 h as described in the Methods section. Each result is the mean ± s.e.mean from several experiments on different lungs (the number indicated by *n*) except for that which refers to cromoglycate pre-incubation for 5 min; this value was calculated from 4 replicates of the same lung. Antigen-induced histamine release in this series of experiments was 13.6 ± 2.6% (range 5.4–30.7%).

This concentration of propranolol had no effect on histamine release when added alone. Similarly, propranolol completely blocked the effect of salbutamol when both drugs were continuously present for 19 h before challenge (Table 2). When added 5 min before challenge to lung fragments which had been pre-incubated for 19 h with salbutamol propranolol reduced the activity of salbutamol by approximately 40% (Table 2).

To confirm that the inhibitory action of salbutamol was mediated through β-adrenoceptors, two experiments were performed in which lung fragments were incubated for 2.5 min with increasing doses (0.1–1000 nM) of (±)-propranolol or (+)-propranolol, before salbutamol addition. In these experiments, salbutamol produced a mean inhibition of antigen-induced histamine release of 52.3 ± 11.0%. The concentrations of (±)- and (+)-propranolol required to produced 50% antagonism of the salbutamol response, as calculated from the best fit regression lines of the two dose-response curves, were 3.0 nM and 452 nM, respectively. This indicated that the racemate of propranolol was approximately 150 times as potent as the (+)-isomer at antagonizing the action of salbutamol.

Characteristics of chlorpromazine inhibition

Chlorpromazine, 100 μM, did not significantly inhibit histamine release from lung fragments when added 30 s before antigen in 5 experiments but was effective when pre-incubated for longer times (Figure 2c). However, when incubated with fragments for periods longer than 15 min, this concentration of chlorpromazine significantly increased the spontaneous release of histamine. For example, in three experiments, incubation of fragments with chlorpromazine 100 μM for 1 h caused spontaneous release to rise from 1.4 ± 0.5% to 4.5 ± 1.2%. Consequently, estimates of inhibition in 1 h and 19 h samples were complicated by the fact that some histamine had been lost from the tissue before antigen challenge was performed. Neither the effects of 5 min nor 19 h pre-incubation could be reversed by thorough washing of the tissue before challenge (Table 1).

Cross-tolerance between sodium cromoglycate and other drugs

Following 19 h pre-incubation with 200 μM sodium cromoglycate, the effects produced by acute addition

Table 2 The effect of propranolol on salbutamol-induced inhibition of anaphylactic histamine release in human lung fragments

Pre-incubation time with propranolol (1 μM)	*Pre-incubation time with salbutamol* (1 μM)	*Inhibition of histamine release (%) Expt. 1*	*Expt. 2*
—	19 h	88.2 ± 10.2	26.1 ± 2.1
19 h*	19 h	−5.1 ± 8.9	−7.2 ± 6.8
5 min**	19 h	53.2 ± 11.2	14.3 ± 14.2

Each result is the mean ± s.e.mean of 4 replicates compared with 6 control replicates incubated with antigen in the absence of drug. Antigen-induced histamine release was 13.9 ± 0.5% in Expt. 1 and 25.7 ± 1.1% in Expt. 2.

* Propranolol was added 2.5 min before salbutamol.

** Propranolol was introduced after the wash stage, before antigen challenge.

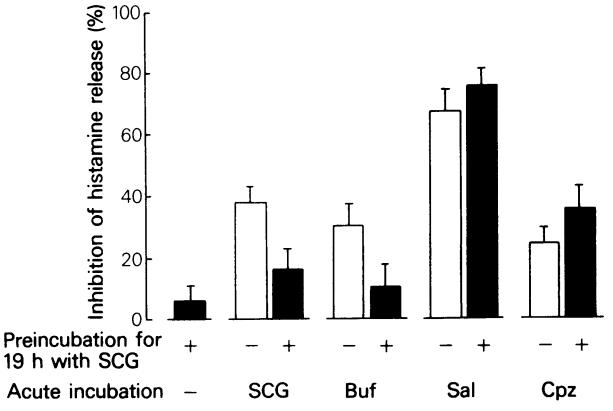

Figure 3 Effect of cromoglycate tolerance on the activities of bufrolin, salbutamol and chlorpromazine. Lung fragments were pre-incubated for 19 h with (solid columns), or without (open columns), sodium cromoglycate 200 μM. Acute treatments were: none; sodium cromoglycate (SCG) 200 μM, 30 s; bufrolin (Buf) 10 μM, 30 s; salbutamol (Sal) 1 μM, 5 min; chlorpromazine (Cpz) 100 μM, 5 min before challenge. Each result is the mean of 7–17 experiments; vertical lines indicate s.e.mean. Antigen-induced histamine release over the whole series was 14.8 ± 1.2% (Range = 6.5–33.3%).

of sodium cromoglycate (200 μM), and the related compound, bufrolin (100 μM), were significantly reduced whereas those of salbutamol (0.1 μM), and chlorpromazine (100 μM), were slightly, but significantly enhanced (Figure 3). The enhancement of salbutamol and chlorpromazine activity was approximately equivalent to the residual effect of the 19 h pre-incubation with sodium cromoglycate.

Discussion

Histamine release from passively sensitized human lung fragments induced by Timothy pollen antigen was strongly inhibited by salbutamol but only weakly by sodium cromoglycate and chlorpromazine. A concentration- and time-related tolerance developed to the effects of sodium cromoglycate but not to those of salbutamol or chlorpromazine. In tissues desensitized to sodium cromoglycate, cross-tolerance was observed only with the related drug bufrolin. These findings suggest that, although all three drugs investigated are capable of preventing immunological mediator release from human mast cells, the mechanisms by which they do so are different.

The inability of sodium cromoglycate to inhibit antigen-induced histamine release totally, even when added in concentrations of up to 200 μM, confirms the findings of previous reports on the activity of this drug in human lung (Sheard & Blair, 1970; Morr, 1978; Sharpe *et al.*, 1978; Butchers *et al.*, 1979; Church & Gradidge, 1980b; Young & Church, 1983). This observation also verifies that sodium cromoglycate is less effective in inhibiting degranulation of human mast cells than it is of rat mast cells (Thomson & Evans, 1973; Orr, 1977).

We have shown that, like rat tissues, human lung fragments become tolerant to the inhibitory effects of sodium cromoglycate following prolonged pre-incubation with the drug before immunological challenge. In several respects, the characteristics of tolerance, or tachyphylaxis, in the two species appear to be similar: the rate of onset and severity of tolerance depends on the concentration of the drug used (Thomson & Evans, 1973; Sung, Saunders, Lenhardt & Chakrin, 1977b); its development is independent of extracellular calcium (Sung, Saunders, Krell & Chakrin, 1977a); once it has developed, recovery from tolerance may not be readily reversed by removal of the drug (Sung *et al.*, 1977b); cross-tolerance develops to drugs with a similar mechanism of action to cromoglycate, such as bufrolin (Marshall *et al.*, 1976). However, the rate of onset of tolerance is different. In the rat, tissue desensitization occurs very rapidly reaching a maximum within 5–10 min (Thomson & Evans, 1973; Sung *et al.*, 1977b) whereas in human lung fragments it develops slowly over several hours.

To explain the mechanism of tolerance to cromoglycate in rat mast cells, the following possibilities have been proposed: (a) depletion of an unidentified labile factor whose endogenous release is promoted by cromoglycate and which normally mediates the inhibitory effect of the drug (Kusner *et al.*, 1973; Thomson & Evans, 1973); the evidence for this concept has since been refuted (Sung *et al.*, 1977a); (b) compensatory, intracellular activation of a phosphodiesterase isoenzyme which is insensitive to cromoglycate (Taylor, Francis, Sheldon & Roitt, 1974); (c) loss or modification of receptor sites on, or in, the mast cell (Marshall *et al.*, 1976; Sung *et al.*, 1977b). The results of our studies suggest that such changes, if they occur in human lung, exhibit different kinetics, i.e. loss of membrane receptors for cromoglycate or stimulation of a cromoglycate-insensitive phosphodiesterase occurs more gradually in human lung cells.

In agreement with previous reports (Butchers *et al.*, 1979; Young & Church, 1982), salbutamol was found to be both more potent and more effective than sodium cromoglycate at inhibiting histamine release from human lung fragments. That salbutamol exerted its effect through stimulation of β-adrenoceptors is supported by the demonstration that (±)-propranolol was some 150 times more potent in blocking salbutamol inhibition than (+)-propranolol; it is known from other pharmacological

systems that β-adrenoceptor-mediated events are 60–100 times more sensitive to blockade by the racemate of propranolol than by its (+)-isomer whereas other effects of the drug are not stereospecific (Barrett & Cullum, 1968; Howe & Shanks, 1969). Butchers, Skidmore, Vardey & Wheeldon (1980) suggest it is the β_2-adrenoceptor which is the target of salbutamol activity. A recent observation that β-stimulant drugs inhibit anaphylaxis in suspensions of human lung mast cells more than 98% pure (Newball and Lichtenstein, personal communication) suggests that salbutamol acts directly on β-adrenoceptors on mast cells rather than indirectly, via release of an inhibitor from another cell-type (Taylor & Sheldon, 1977).

Tolerance to the effects of β-adrenoceptor stimulants has been demonstrated in many cell-types, e.g. guinea-pig macrophages (Remold-O'Donnell, 1974), frog erythrocytes (Mukherjee, Caron & Lefkowitz, 1975), and human bronchial smooth muscle (Davis & Conolly, 1980), the desensitization sometimes taking up to 24 h to develop fully (Mukherjee *et al.*, 1975). One proposed mechanism of this desensitization is uncoupling of β-adrenoceptors from adenylate cyclase and internalisation of the agonist-receptor complex by the cell (Harden, Cotton, Waldo, Lutton & Perkins, 1980). Furthermore, resistance can occur to the bronchodilator effects of β-stimulants *in vivo* (Holgate, Baldwin & Tattersfield, 1977), although this is not demonstrable in subjects with asthma or atopy (Harvey & Tattersfield, 1982), nor is the phenomenon necessarily related to the tolerance seen at the cellular level (Paterson, Woolcock & Shenfield, 1980). In human lung fragments, we failed to observe any tolerance to the anti-anaphylactic effect of salbutamol, even when the tissue was exposed to drug for up to 19 h before challenge. Similarly, Grönneberg & Strandberg (1981) did not detect any tolerance when the β-stimulant terbutaline was injected intradermally into human skin, up to 8 h before antigen challenge. These results suggest that, unlike several other cell-types, human mast cells do not develop tolerance to β-stimulant drugs.

The effects of short pre-incubation periods with salbutamol were, like those of sodium cromoglycate, readily reversed by washing the tissue free of drug before challenge. Thus, the binding of salbutamol to lung cells is likely to be weak and the sequelae of short term exposure may be transient. However, the nature of the interaction between salbutamol and the lung mast cells appeared to change with prolonged incubation. After 19 h pre-incubation, its effects were not reversed by washing and only partially blocked by the late addition of propranolol. The reasons for this are unclear and require further investigation.

Unlike either sodium cromoglycate or salbutamol, chlorpromazine had a slow onset of action, being ineffective when added 30 s before antigen challenge and slowly increasing in efficacy over 1 h. The finding that on 5 min pre-incubation it was only a partial inhibitor of histamine release supports our previous observations (Church & Gradidge, 1980a; Young & Church, 1983). Since the inhibitory effects of chlorpromazine could not be reversed by washing the lung tissue before challenge, it is likely that the drug binds strongly to, or is taken up by, mast cells in the lung, due to either its lipophilicity (Seeman, 1972) or its propensity for binding to proteins (Rosen & Tham, 1980). At high concentrations and following prolonged pre-incubation with lower concentrations, chlorpromazine induced release of histamine in the absence of antigen challenge, probably by a cytotoxic mechanism involving rupture of the mast cell plasmalemma (Seeman, 1966; Frisk-Holmberg, 1972). This release of histamine may have given an artificially high indication of the ability of the drug to stabilize mast cells following prolonged pre-incubation for 19 h.

In addition to the concentration- and time-related differences in the inhibitory effects of sodium cromoglycate, salbutamol and chlorpromazine described above, the lack of cross-tolerance between cromoglycate and the other two compounds increases the likelihood that the three drugs act on lung mast cells via totally different mechanisms. Sodium cromoglycate inhibition in human lung in many ways resembles its inhibition in rats. However, its partial activity and the slower development of tolerance, both evident at concentrations much higher than those likely to be achieved in the lung during asthma therapy, suggest that human mast cells are less sensitive to the drug than those of the rat. The finding that salbutamol is a potent and effective inhibitor of anaphylactic histamine release in human lung and that exposure to the drug for long periods does not induce tolerance, indeed, the activity of the drug seems to become less easily reversible, may both contribute to its beneficial effects in the chronic treatment of asthma. Chlorpromazine only effectively inhibits mediator release from human lung at a concentration well in excess of doses tolerated in man and, as prolonged exposure of mast cells to this concentration causes release of histamine, the therapeutic value of this and related drugs in allergic disease may be limited.

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References

ASSEM, E.S.K. & SCHILD, H.O. (1969). Inhibition by sympathomimetic amines of histamine release induced by antigen in passively sensitised human lung. *Nature*, **224**, 1028–1029.

BARRETT, A.M. & CULLUM, V.A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BUTCHERS, P.R., FULLARTON, J.R., SKIDMORE, I.F., THOMSON, L.E., VARDEY, C.J. & WHEELDON, A. (1979). A comparison of the anti-anaphylactic activities of salbutamol and disodium cromoglycate in the rat, the rat mast cell and in human lung tissue. *Br. J. Pharmac.*, **67**, 23–32.

BUTCHERS, P.R., SKIDMORE, I.F., VARDEY, C.J. & WHEELDON, A. (1980). Characterisation of the receptor mediating the anti-anaphylactic effects of β-adrenoceptor agonists in human lung tissue *in vitro*. *Br. J. Pharmac.*, **71**, 663–667.

CAULFIELD, J.P., LEWIS, R.A., HEIN, A. & AUSTEN, K.F. (1980). Secretion in dissociated human pulmonary mast cells. Evidence for solubilisation of granule contents before discharge. *J. cell. Biol.*, **85**, 299–311.

CHURCH, M.K. & GRADIDGE, C.F. (1980a). Inhibition of histamine release from human lung *in vitro* by antihistamines and related drugs. *Br. J. Pharmac.*, **69**, 663–667.

CHURCH, M.K. & GRADIDGE, C.F. (1980b). The activity of sodium cromoglycate analogues in human lung *in vitro*: a comparison with rat passive cutaneous anaphylaxis and clinical efficacy. *Br. J. Pharmac.*, **70**, 307–311.

DAVIS, C. & CONOLLY, M.E. (1980). Tachyphylaxis to β-adrenoceptor agonists in human bronchial smooth muscle: studies *in vitro*. *Br. J. clin. Pharmac.*, **10**, 417–423.

DONLON, M.A., HUNT, W.A., CATRAVAS, G.N. & KALINER, M. (1980). Identification of β-adrenergic receptors on rat peritoneal mast cell granule membranes. *Fedn Proc.*, **39**, 643.

FRISK-HOLMBERG, M. (1972). Drug-induced changes in release and uptake of biogenic amines. *Acta physiol. scand*; suppl., **376**, 1–36.

GRÖNNEBERG, R. & STRANDBERG, K. (1981). Effect of terbutaline on cutaneous responses in man to rechallenge with allergen and compound 48/80. *Allergy*, **36**, 33–38.

HARDEN, T.K., COTTON, C.U., WALDO, G.L., LUTTON, J.K. & PERKINS, J.P. (1980). Catecholamine-induced alteration in sedimentation behaviour of membrane bound β-adrenergic receptors. *Science*, **210**, 441–443.

HARVEY, J.E. & TATTERSFIELD, A.E. (1982). Airway response to salbutamol: effect of regular salbutamol inhalations in normal atopic and asthmatic subjects. *Thorax*, **37**, 280–287.

HERZIG, D.J., KUSNER, E.J., FOX, J. & KAPLAN, H.R. (1977). Relationship between "Bezold-Jarisch" effect in dogs and anti-allergy action of cromolyn-type compounds? *Fedn Proc.*, **36**, 987.

HOLGATE, S.T., BALDWIN, C.J. & TATTERSFIELD, A.E. (1977). β-adrenergic agonist resistance in normal human airways. *Lancet*, **ii**, 375–377.

HOWE, R. & SHANKS, R.G. (1969). Optical isomers of propranolol. *Nature*, **210**, 1336–1338.

ISHIZAKA, T. (1981). Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. *J. Allergy clin. Immunol.*, **67**, 90–96.

JOHNSON, A.R. & MORAN, N.C. (1970). Inhibition of the release of histamine from rat mast cells: the effect of cold and adrenergic drugs on the release of histamine by compound 48/80 and antigen. *J. Pharmac. exp. Ther.*, **175**, 632–640.

KUSNER, E.J., DUBNICK, B. & HERZIG, D.J. (1973). The inhibition by disodium cromoglycate *in vitro* of anaphylactically induced histamine release from rat peritoneal mast cells. *J. Pharmac. exp. Ther.*, **184**, 41–46.

LICHTENSTEIN, L.M., FOREMAN, J.C., CONROY, M.C., MARONE, G. & NEWBALL, H.H. (1979). Differences between histamine release from rat mast cells and human basophils and mast cells. In *The Mast Cell – Its Role in Health and Disease*. ed. Pepys, J. & Edwards, A.M. pp. 83–96. London: Pitman Medical.

MARSHALL, P.W., THOMSON, D.S. & EVANS, D.P. (1976). The mechanism of tachyphylaxis to ICI 74,917 and Disodium Cromoglycate. *Int. Archs Allergy appl. Immun.*, **51**, 274–283.

MORR, H. (1978). Effect of disodium cromoglycate on antigen-induced histamine release from passively sensitised human lung *in vitro*. *Lung*, **155**, 33–38.

MOTA, I. & DIAS DA SILVA, W. (1960). The anti-anaphylactic and histamine-releasing properties of the anti-histamines: their effect on mast cells. *Br. J. Pharmac. Chemother.*, **15**, 396–404.

MUKHERJEE, C., CARON, M.G. & LEFKOWITZ, R.J. (1975). Catecholamine-induced subsensitivity of adenylate cyclase associated with loss of β-adrenergic receptor binding sites. *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1945–1949.

ORR, T.S.C. (1977). Mode of action of disodium cromoglycate. *Acta Allergologica*, suppl., **13, 32**, 9–27.

PATERSON, J.W., WOOLCOCK, A.J. & SHENFIELD, G.M. (1980). Bronchodilator drugs. *Amer. Rev. Resp. Dis.*, **120**, 1149–1188.

REMOLD-O'DONNELL, E. (1974). Stimulation and desensitisation of macrophage adenylate cyclase by prostaglandins and catecholamines. *J. biol. Chem.*, **249**, 3615–3621.

ROSEN, A. & THAM, S.Y. (1980). The binding of chlorpromazine to some fractions of homogenised rat brain. *J. Pharm. Pharmac.*, **32**, 357–359.

SEEMAN, P. (1966). Erythrocyte membrane stabilisation by local anaesthetics and tranquillisers. *Biochem. Pharmac.*, **15**, 1753–1766.

SEEMAN, P. (1972). The membrane actions of anaesthetics and tranquillisers. *Pharmac. Rev.*, **24**, 583–655.

SHARPE, T. J., ROSS, J.W. & SPICER, B.A. (1978). The effect of sodium 5,6-dimethyl-2-nitroindanedione on anaphylactic reactions *in vitro*. *Agents and Actions*, **8**, 199–202.

SHEARD, P. & BLAIR, A.M.J.N. (1970). Disodium cromoglycate: activity in three *in vitro* models of the immediate hypersensitivity reaction in lung. *Int. Archs Allergy appl. Immun.*, **38**, 217–224.

SUNG, C-P., SAUNDERS, H.L., KRELL, R.D. & CHAKRIN, L.W. (1977a). Studies on the mechanism of tachyphylaxis to disodium cromoglycate. *Int. Archs Allergy appl. Immun.*, **55,** 374–384.

SUNG, C-P., SAUNDERS, H.L., LENHARDT, E. & CHAKRIN, L.W. (1977b). Further studies on the mechanism of tachyphylaxis to DSCG. The effects of concentration and temperature. *Int. Archs Allergy appl. Immun.*, **55,** 385–394.

TAYLOR, W.A., FRANCIS, D.H., SHELDON, D. & ROITT, I.M. (1974). Anti-allergic actions of disodium cromoglycate and other drugs known to inhibit cyclic 3′,5′-nucleotide phosphodiesterase. *Int. Archs Allergy appl. Immun.*, **47,** 175–193.

TAYLOR, W.A. & SHELDON, D. (1977). Effect of lung tissue on the inhibitory actions of isoprenaline, theophylline, disodium cromoglycate and PGE_1 on antigen induced mast cell degranulation. *Int. Archs Allergy appl. Immun.*, **54,** 322–328.

THOMSON, D.S. & EVANS, D.P. (1973). Inhibition of immediate hypersensitivity reactions by disodium cromoglycate. Requirements for activity in two laboratory models. *Clin. exp. Immunol.*, **13,** 537–544.

YOUNG, K.D. & CHURCH, M.K. (1980). Tolerance to sodium cromoglycate in human lung fragments *in vitro*. *Br. J. Pharmac.*, **80,** 122–123P.

YOUNG, K.D. & CHURCH, M.K. (1983). Passive anaphylaxis in human lung fragments as a model for testing anti-allergic drugs: its variability and constraints. *Int. Archs Allergy appl. Immun.*, (in press).

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