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EFFECTS OF CYCLIC AMP- AND CYCLIC GMP-PHOSPHODIESTERASE INHIBITORS ON IMMUNOLOGICAL RELEASE OF HISTAMINE AND ON LUNG CONTRACTION

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1 Cyclic adenosine 3′,5′-monophosphate (cyclic AMP)- and cyclic guanosine 3′,5′-monophosphate (cyclic GMP)-phosphodiesterase activities from rat lung were selectively inhibited by ZK 62711 and M&B 22948, respectively. Theophylline and papaverine inhibited both activities.

2 Rat lung strips contracted by carbachol were relaxed by 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62711, $EC_{25} = 7 \times 10^{-8}$M) and 2-O-propoxyphenyl-8-azapurin-6-one (M&B 22948, $EC_{25} = 5 \times 10^{-7}$M) indicating relaxant properties of both cyclic AMP and cyclic GMP.

3 The antigen-induced histamine release from human basophils was inhibited by ZK 62711 ($IC_{25} = 8 \times 10^{-7}$M), whereas M&B 22948 had no effect. On the contrary, the release from rat mast cells was inhibited by M&B 22948 ($IC_{25} = 10^{-6}$M), while ZK 62711 had no effect.

4 These data show an inhibitory effect of cyclic AMP on histamine release to be involved with basophils, whereas cyclic GMP is predominantly involved with mast cells. It is suggested that the antianaphylactic properties of cyclic nucleotide phosphodiesterase inhibitors are mainly linked to the increase of cyclic GMP.

Introduction

Anti-asthmatic properties have been attributed to cyclic nucleotide phosphodiesterase inhibitors. These properties might be related to both inhibition of secretion of mediators of anaphylaxis and relaxation of lung smooth muscle, two cyclic nucleotide-dependent phenomena (for a review, see Gold, 1980). The concept that increases in cyclic adenosine 3′,5′-monophosphate (cyclic AMP) reduce mediator release and increases in cyclic guanosine 3′,5′-monophosphate (cyclic GMP) enhance release was introduced by Kaliner, Orange & Austen (1972). Similarly, it was suggested that cyclic AMP acted as a relaxing factor of lung smooth muscle and cyclic GMP as a contracting factor (Kuo & Kuo, 1973; Murad & Kimura, 1974). According to these hypotheses, phosphodiesterase inhibitors should be considered as anti-anaphylactic agents through the increase of cyclic AMP levels. However, as pointed out in a recent review by Conolly (1980), these concepts require re-evaluation, as the increase of cyclic GMP levels could also be implicated in the anti-asthmatic properties of phosphodiesterase inhibitors. The present paper describes the effects of 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62711), a selective inhibitor of cyclic AMP phosphodiesterase and of 2-O-propoxyphenyl-8-azapurin-6-one (M&B 22948), a selective inhibitor of cyclic GMP phosphodiesterase, on the contraction of rat lung strips and on the antigen-induced histamine release from rat mast cells and human basophils, compared to theophylline, a non-selective inhibitor of cyclic AMP and cyclic GMP hydrolysis.

Methods

Relaxation of rat lung strips

Male Sprague-Dawley rats weighing from 250 to 300 g were anaesthetized with 50 mg/kg sodium pentobarbitone injected intraperitoneally. Lung strips were dissected as described by Lulich, Mitchel & Sparrow (1976). The strips were placed in 10 ml organ baths containing modified Krebs solution aerated with 95% O_2 and 5% CO_2, at 37°C and had a tension of 0.4 g applied. The tissues were allowed to equilibrate for 2 h, the solution being changed at 15 min intervals. Changes in tension were measured isometrically with a Narco Biosystem F60 myograph connected to a Narco recorder. Concentration-response relationships of inhibitors were determined with a cumulative dose schedule (Van Rossum, 1963), after contraction of lung strips with 2×10^{-5}M carbachol (see Figure 1).

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Inhibition of phosphodiesterase activities

Lung strips were prepared from 7 rats as described above and homogenized with 8 ml of a buffer containing $MgCl_2$ 2 mM, EGTA (ethylene bis (oxyethylenenitrilo)-tetraacetic acid) 0.1 mM, and Tris-HCl pH 7.5 20 mM, using a glass Potter homogenizer at 4°C. The homogenate was centrifuged at 105,000 *g* for 60 min. The supernatant was collected and stored at −80°C. Phosphodiesterase activities were determined according to Keravis, Wells & Hardman (1980) in the presence of $MgCl_2$ 2 mM, EGTA 0.02 mM, cyclic AMP 1 μM or cyclic GMP and Tris-HCl 20 mM pH 7.5. Specific activities of the preparation were 271 and 728 pmol of cyclic AMP and cyclic GMP hydrolyzed min^{-1} mg^{-1} protein. IC_{50}s (concentrations of the compounds required to give 50% inhibition of phosphodiesterase activity) were determined by interpolations of at least 4 values of inhibition (means of duplicates) ranging from 35 to 75% against the logarithm of inhibition concentrations. Inhibitors were dissolved in the presence of dimethylsulphoxide; aliquots were stored at −20°C and thawed immediately before use. No analytical interference of inhibitors or solvents was observed under the conditions used.

Histamine release from rat peritoneal mast cells

Male Wistar rats weighing 300 g were sensitized according to Jarret (1978) using an intraperitoneal injection of 0.3 ml of 2% $Al(OH)_3$ suspension including 20 μg ovalbumin; 10 to 14 days later rats were killed by decapitation and exsanguinated. Phosphate buffer (10 ml) was injected into the peritoneal cavity. The body was gently massaged for 1 min and the peritoneal fluid collected and centrifuged for 2 min at 220 *g*. The cellular pellet was resuspended in the phosphate buffer to obtain about 20,000 mast cells/ml; 0.9 ml of the cellular suspension was preincubated for 20 min at 37°C in plastic tubes with 50 μl of buffer including drugs. The histamine release was induced by addition of 0.1 mg/ml of ovalbumin. After 15 min incubation at 37°C the tubes were cooled at 4°C and centrifuged at 220 *g* for 7 min. Supernatants were collected and histamine concentrations measured according to the fluorimetric method of Shore, Burkhalter & Cohn (1959) with a continuous flow automated device. The spontaneous release of histamine determined in the absence of ovalbumin was 2 to 4% of the total cellular histamine content measured for each sample after treatment of cell suspension with perchloric acid. All the determinations were performed in duplicate. No analytical interference of drugs with the histamine measurement was observed under the conditions used.

Histamine release from human basophils

Blood was drawn from asthmatic patients with positive skin test to *Dermatophagoides pteronyssinus* and leucocytes were separated by the dextran sedimentation procedure described by Lichtenstein & Osler (1964). Cells were washed twice in buffer A and resuspended in buffer B (see below) to obtain 3×10^6 cells/ml, corresponding to 2 to 4×10^4 basophils/ml. The trypan blue exclusion test showed 98% intact cells. Cells were preincubated for 20 min at 37°C with or without drugs. Preliminary experiments had shown this time to be optimal for maximum inhibition by ZK 62711. Histamine release was induced by adding 0.1 μg/ml of *Dermatophagoides pteronyssinus* extract, corresponding to 80 to 100% of total histamine content as determined for each sample by preliminary dose-response curves. After 40 min incubation at 37°C the reaction was stopped at 4°C and histamine measured as described for rat mast cells with similar controls.

Chemicals and buffers

M&B 22948 (2-O-propoxyphenyl-8-azapurin-6-one) was a gift from May and Baker, ZK 62711 [Rolipram, 4-(3-cyclopentyloxy-4-methoxy-phenyl)-2-pyrrolidone] from Schering, papaverine from Synthelabo; disodium cromoglycate (DSCG) from Fisons; and *Dermatophagoides pteronyssinus* extract from Institut Pasteur. Theophylline was obtained from Merck; carbachol and ovalbumin grade V from Sigma; and 2′-deoxy-cyclic AMP and 2′-deoxy-cyclic GMP from Boehringer-Mannheim. All other chemicals were of analytical grade. Buffer A contained 120 mM NaCl, 5 mM KCl, 0.3 mg/ml human serum albumin (Calbiochem) and 25 mM trishydroxymethylaminomethane HCl pH 7.6. Buffer B was prepared as buffer A but included 0.6 mM $CaCl_2$ and 1 mM $MgCl_2$. Phosphate buffer contained (mM): NaCl 150, KCl 3.7, $CaCl_2$ 0.9, Na_2HPO_4 3.5 and KH_2PO_4 3, pH 7.2. The modified Krebs buffer contained (mM): NaCl 118, KCl 4.75, $CaCl_2$ 1.25, $MgSO_4$ 1.2, glucose 10, KH_2PO_4 1.15 and $NaHCO_3$ 10 (pH 7.5).

Results

Table 1 shows the inhibition of cyclic AMP- and cyclic GMP-phosphodiesterase activities from rat lung 105,000 *g* supernatant by various drugs. The hydrolysis of cyclic AMP was strongly inhibited by ZK 62711 and 2′deoxy-cyclic AMP, whereas M&B 22948, 2′deoxy-cyclic GMP, and DSCG selectively inhibited the hydrolysis of cyclic GMP.

Table 1 Inhibition of cyclic AMP and cyclic GMP phosphodiesterase activities from 105,000 *g* supernatant of rat lung strips (see Methods)

Inhibitors	*Potencies IC_{50} (μM)*		*Selectivities*
	Cyclic AMP (a)	*Cyclic GMP* (b)	$(\frac{b}{a})$
2'Deoxy-cyclic AMP	8	1200	150
ZK 62711	25	300	12
Theophylline	180	290	1.6
Papaverine	35	50	1.4
DSCG	> 2500	230	< 0.09
2'Deoxy-cyclic GMP	440	1.5	0.003
M&B 22948	210	0.7	0.003

DSCG = disodium cromoglycate.

Papaverine and theophylline inhibited both activities. In the following experiments, ZK 62711 and M&B 22948 were chosen as selective inhibitors of cyclic AMP- and cyclic GMP-phosphodiesterase, respectively.

Rat lung strips were contracted with carbachol. 5-Hydroxytryptamine produced a smaller contraction than carbachol and histamine had no effect (Figure 1). Figure 2 shows that both ZK 62711 and M&B 22948 were able to relax lung strips contracted with carbachol, with EC_{25}s of 7×10^{-8}M and 5×10^{-7}M, respectively. Isoprenaline was more potent ($EC_{25} = 5 \times 10^{-9}$M). High concentrations of theophylline were necessary to induce relaxation ($EC_{25} = 4 \times 10^{-5}$M).

The effect of these phosphodiesterase inhibitors was studied on the antigen-induced release of histamine. Figure 3 shows that ZK 62711 lowered the dose-dependent antigen-induced release of histamine from human leucocytes and that M&B 22948 had a similar effect on rat mast cells. Figure 4 shows that in the presence of an optimal concentration of antigen, ZK 62711 was highly active, but M&B 22948 completely inactive, in inhibiting release from human leucocytes and conversely for rat mast cells. Theophylline was weakly active on both preparations.

Discussion

The modulation of mediator release by cyclic AMP was first suggested by Lichtenstein & Margolis (1968). They showed that catecholamines and methylxanthines inhibited the antigen-induced release from human leucocytes, both types of drugs increasing cyclic AMP levels (Butcher & Sutherland 1962). Assem & Schild (1969) and Ishizaka, Ishizaka, Orange & Austen (1970) reached similar conclusions concerning release of histamine and slow-reacting substance of anaphylaxis (SRS-A) from human and monkey lung. The understanding of

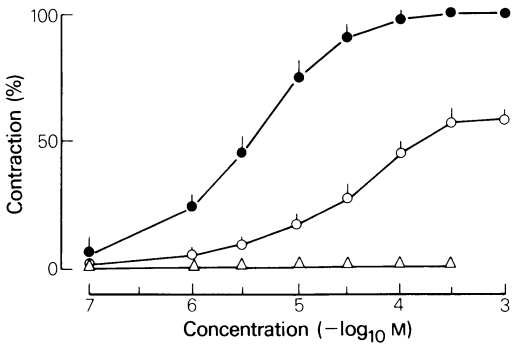

Figure 1 Concentration-response curves to carbachol (●), 5-hydroxytryptamine (○) and histamine (△) on the rat isolated lung strip. The contractions are expressed as a percentage (mean of 6 experiments) of the maximal contraction produced by carbachol; vertical bars indicate s.e.mean.

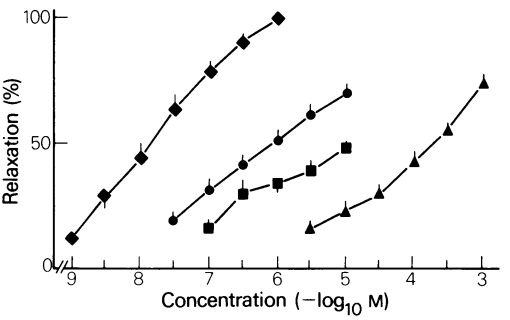

Figure 2 Concentration-response curves to isoprenaline (◆), ZK 62711 (●), M&B 22948 (■) and theophylline (▲) on the rat isolated lung strip previously contracted with 2×10^{-5}M carbachol. The relaxations are expressed as a percentage (means of 6 experiments) of the maximal relaxation produced by isoprenaline.

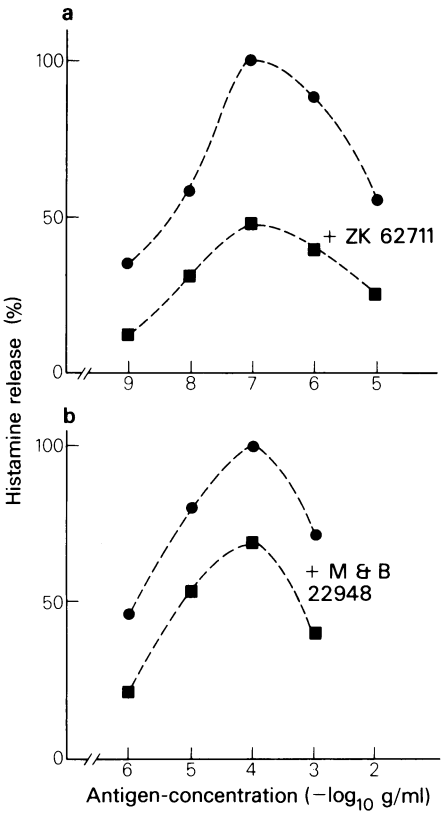

Figure 3 Concentration-response curves to antigen on histamine release from human basophils (a) and rat mast cells (b) in the presence (■) or absence (●) of 10^{-5}M ZK 62711 or M&B 22948. Histamine release was induced by additing various concentrations of *Dermatophagoides pteronyssinus* extract to human basophils or egg albumin to rat mast cells as described in Methods.

this phenomenon was enhanced when Orange, Kaliner, Laraia & Austen (1971) showed that these inhibitions were related to the degree of elevation of tissue levels of cyclic AMP. The converse situation, namely the enhancement of mediator release by cholinergic stimulation or analogues of cyclic GMP was reported by Kaliner *et al.* (1972) and Kaliner (1977). These various observations led to the concept that increases in cyclic AMP reduce mediator release and increases in cyclic GMP enhance release. However, Coulson, Ford, Marshall, Walker, Wooldridge, Bowden & Coombs (1977) showed that the inhibition of immunological histamine release from human lung and passive cutaneous anaphylaxis in the rat were correlated to the relative ability of drugs to inhibit cyclic GMP-hydrolysis. The observation that M&B 22948, a selective inhibitor of cyclic GMP phosphodiesterase (Table 1), inhibited histamine release from mast cells (Figure 4) is in agreement with these latest data. M&B 22948 was indeed introduced as an inhibitor of reagin-mediated anaphylaxis (Broughton, Chaplen, Knowles, Lunt, Pain, Wooldridge, Ford, Marshall, Walker & Maxwell, 1974). Similarly ICI 74917 inhibits immediate hypersensitivity reactions (Evans & Thomson, 1975), and selectively inhibits cyclic GMP hydrolysis (Coulson *et al.*, 1977; Ruckstuhl & Landry, 1981). It must also be noted that DSCG is a highly selective inhibitor of cyclic GMP hydrolysis (Bergstrand, Kristofferson, Lundquist & Schurmann 1977; and Table 1) and has been previously shown to inhibit histamine release from mast cells but not from basophils (Assem & Mongar, 1970). The structure-activity relationship of these different chromone-like compounds have recently been studied (Ruckstuhl, Beretz, Anton & Landry, 1979; Ruckstuhl & Landry, 1981). The study of Coulson *et al.* (1977) mentioned above, did not include inhibitors with high selectivity for cyclic AMP hydrolysis. In the present work, we show that ZK 62711, a selective inhibitor of cyclic AMP hyd-

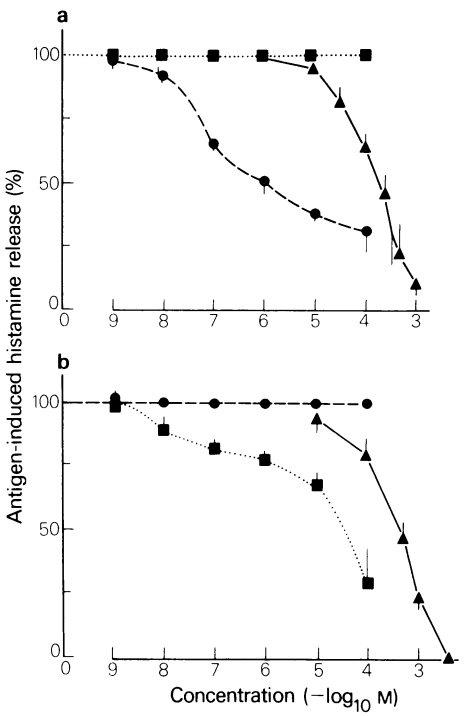

Figure 4 Concentration-response curves to ZK 62711 (●), M&B 22948 (■), and theophylline (▲) on the antigen-induced release of histamine from human basophils (a) and rat mast cells (b). Histamine release is expressed as a percentage (means of 10 experiments) of the release observed in the absence of inhibitors; vertical bars indicate s.e.mean.

rolysis (Schwabe, Miyake, Ohga & Daly, 1976 and Table 1), has no effect on the histamine release from mast cells. Consequently it can be concluded that, instead of cyclic AMP, increases of cyclic GMP are involved in the inhibition of histamine release by phosphodiesterase inhibitors in rat mast cells, confirming the correlation obtained by Coulson *et al.* (1977) on human lung. It has to be considered that the experiments which first suggested cyclic AMP as a negative factor in histamine release were performed on human leucocytes (Lichtenstein & Margolis, 1968), which is what led us to use such cells. Indeed in this case, ZK 62711 inhibited antigen-induced histamine release, whereas M&B 22948 had no effect. Consequently, it may be suggested that the modulation of mediator release is different from one cell type to another, increase of cyclic AMP levels leading to the inhibition of histamine release in basophils, while in mast cells, cyclic GMP appears to be predominantly involved. It should be pointed out that in both rat mast cells (Sullivan, Parker, Kulczycki & Parker, 1976) and human basophils (Lichtenstein, Sobotka, Malveaux & Gillespie, 1978), antigen stimulation induced a peak of cyclic AMP followed by a rapid decrease. However cyclic GMP has not been measured in such experiments.

Inhibitors of cyclic AMP and cyclic GMP hydrolysis are able to induce relaxation of lung smooth muscle (Figure 2) as recently found by Fredholm, Brodin & Strandberg (1979) in guinea-pig smooth muscle. The role of cyclic AMP as a relaxing mediator is well established (for a review see Gold, 1980) but the role of cyclic GMP is still uncertain. Indeed, it was suggested that cyclic GMP was primarily involved in lung smooth muscle contraction (Kuo & Kuo, 1973; Murad & Kimura, 1974) but experiments on other smooth muscles showed relaxant effects of increased cyclic GMP levels (Diamond & Blisard, 1976; Schultz, Schultz & Schultz, 1977). Moreover Kukovetz, Holzman, Wurm & Pöch (1979) showed that M&B 22948 potentiated the relaxant effects of nitro-compounds in coronary smooth muscle. Altogether, these results suggest that both cyclic AMP and cyclic GMP could be involved in the relaxant effect of phosphodiesterase inhibitors.

In conclusion, we suggest that the antiasthmatic properties of phosphodiesterase inhibitors are linked to the inhibition of mast cell histamine release through cyclic GMP increase, and to lung relaxation through increases of both cyclic AMP and cyclic GMP.

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