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INHIBITION OF cAMP PHOSPHODIESTERASE BY DISODIUM CROMOGLYCATE

ASHIM C. ROY and BRIAN T. WARREN

Research Division, Miles Laboratories Limited, Stoke Poges,

Slough SL2 4LY, England.

Previous studies[1] on the mode of action of disodium cromoglycate (DSCG) have shown that the drug blocks the IgE-mediated release of spasmogens by acting at a stage in the allergic pathway subsequent to antigen-antibody combination, but prior to spasmogen release. It does not antagonize bradykinin, SRS-A or histamine; neither is it a β-adrenergic stimulant. The inhibition by DSCG of non-immunological triggered spasmogen release has also been demonstrated.

Cyclic 3',5'-AMP (cAMP) has been implicated as a regulator of IgE-mediated allergic reactions in various models of immediate hypersensitivity. For example, β-adrenergic stimulants (which stimulate cAMP production), methylxanthines (which inhibit phosphodiesterase activity and prevent cAMP degradation) and dibutyryl cAMP (which mimics endogenous cAMP action) have been shown to inhibit the antigen-induced IgE-mediated release of histamine from human leucocytes[2], human[3,4], monkey[5] and guinea-pig lung[6], and SRS-A release in rat peritoneal anaphylaxis[7]. We have now examined the ability of DSCG to inhibit cAMP phosphodiesterases from several sources.

The enzyme preparations used were human lung homogenate (60% $(NH_4)_2SO_4$ fraction); guinea-pig lung homogenate (3,000g supernatant fraction); bovine heart (Sigma); rat peritoneal cell preparation (3,000g supernatant fraction).

Measurement of cAMP phosphodiesterase activity at 37° for 15 min was based on the method of Huang and Kemp[8]. Samples (1.3ml) contained 50 mM Tris-HCl

(pH 7.5), 10 mM $MgCl_2$, ^{3}H-cAMP (50,000 c.p.m.), 0.08 mM unlabelled cAMP, the enzyme preparation (in 50 mM Tris-HCl, pH 7.5 containing 5 mM 2-mercaptoethanol) with or without drug. Reaction was stopped by immersion in boiling water for 3 min. ^{3}H-5'-AMP formed from ^{3}H-cAMP was converted to ^{3}H-adenosine by *Crotalus atrox* venom (400 µg/tube), separated on DEAE Sephadex A-25 and counted. None of the drugs examined influence the activity of the venom enzyme.

The results presented in Table I show that, with one exception, DSCG is a more potent inhibitor than the standard phosphodiesterase inhibitor, theophylline, especially on the preparation from human lung which is the probable site of DSCG action. Of a variety of anti-inflammatory and anti-allergic drugs that were also examined, aspirin, sodium salicylate, dexamethasone, mepyramine, phenylbutazone, isoprenaline, salbutamol and phenoxybenzamine, only indomethacin (ID_{50} 155µM and 253µM for bovine heart and human lung enzymes, respectively), for which phosphodiesterase inhibitor activity has been reported[9], showed significant activity indicating some degree of specificity for DSCG.

Table I. Effect of DSCG and theophylline on cAMP phosphodiesterase activity from different sources.

Enzyme source	ID_{50}(µM)	
	DSCG	Theophylline
Human lung	380	980
Guinea-pig lung	540	830
Bovine heart	462	470
Rat peritoneal cells	820	1150

The kinetics of the inhibition were investigated with the enzyme from bovine heart; the Dixon[10] plot (Figure 1) shows competitive inhibition for DSCG with an inhibitor constant k_i, 3.0×10^{-4}M, comparable to that for theophylline, k_i 3.3×10^{-4}M.

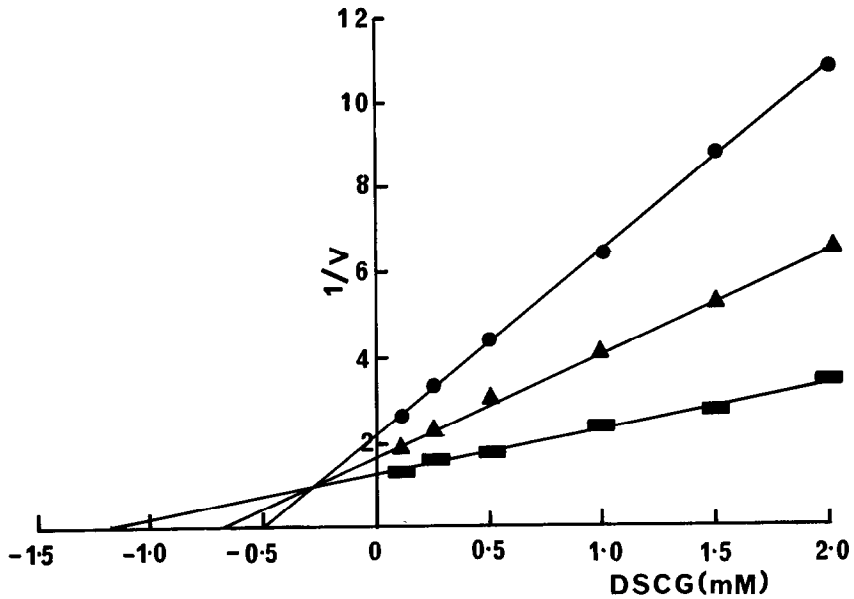

Figure 1. Dixon plot for DSCG on bovine heart cAMP phosphodiesterase. Substrate concentration: -●- 20μM. -▲- 40μM, -■- 80μM.

Reported results of the effect of isoprenaline and theophylline on the inhibition by DSCG of the antigen-induced release of SRS-A in rats prepared with IgE antibody would suggest that, in the system examined, DSCG operates by a mechanism independent of cAMP[7]. Evidence supporting the implication of our *in vitro* results that DSCG operates through a cAMP dependent mechanism by inhibiting cAMP phosphodiesterases is forthcoming from the work of Taylor *et al*[11] of this department. They have found that DSCG and isoprenaline act synergistically in inhibiting both passive cutaneous anaphylaxis in the rat and the degranulation of rat peritoneal mast cells induced by phospholipase-A. One of us had previously examined the suggestion[12] that DSCG blocks histamine release from mast cells by inhibiting phospholipase-A known to be present on the mast cell surface. The negative results support the view[13] that DSCG does not act on this enzyme (A.C. Roy, unpublished).

Recently[14], the existence of phosphodiesterase isoenzymes has been suggested to explain differential drug sensitivity. Further studies to establish the possible selective action of DSCG on phosphodiesterase isoenzymes from various sources are underway.

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