J. Enzyme Inhibition, 1996, Vol. 10, pp. 135–139
Reprints available directly from the publisher
Photocopying permitted by license only

EFFECT OF THE ANTIALLERGIC DRUG DISODIUM CROMOGLYCATE ON PHOSPHODIESTERASE ACTIVITY

EDUARDO OCHOA DE ASPURU and ANA Mª LOURDES ZATÓN*

Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Basque Country, P.O.B. 450 Vitoria-Gasteiz, Spain

(Received 10 April 1995; in final form 24 July 1995)

The antiallergic drug disodium cromoglycate and its non-antiallergic analogue dicoumarol, inhibit the enzymatic activity of phosphodiesterase with K_I values of $0.8 \cdot 10^{-9}$ M and $1.6 \cdot 10^{-9}$ M, respectively. It seems that the dichromone group is responsible for this inhibition, independently of its antiallergic effect.

KEY WORDS: Phosphodiesterase, disodium cromoglycate, inhibition, dicoumarol

INTRODUCTION

Disodium cromoglycate (DSCG) blocks liberation of histamine,[1–7] a vasoactive substance liberated in allergy. The drug neither inhibits IgE binding to these cells nor interaction between binding IgE and a specific antigen, but suppresses secretory response to this interaction, raising intracellular concentration of cAMP.[8] It has been suggested that cromoglycate increases intracellular level of 3′,5′-cyclic guanosine monophosphate (cGMP) by inhibition of suitable phosphodiesterase (PDE) or stimulation of guanylate cyclase.[9] The increase of cyclic nucleotide levels may activate a natural mechanism to stop histamine liberation, based on phosphorylation of a membrane protein by a cyclic nucleotide-sensitive protein kinase.[9–11]

The blockage of the liberation of allergic mediators in the presence of cromoglycate may be due to the increase in cyclic nucleotide levels, as a result of inhibition of PDE activity. We have examined the possible blockage of the liberation of mediators through inhibition of PDE by studying PDE activity in the presence of the antiallergic drug cromoglycate and in the presence of other dichromones and chromones which do not possess antiallergic activity.

MATERIALS AND METHODS

cAMP-dependent phosphodiesterase from bovine heart (EC 3.1.4.17; P 0395) was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

*Correspondence.

Disodium cromoglycate was obtained from Fisons Limited (Loughorough, England); 2H-1-benzopyran-4-one (coumarin), and 3,3-methylene bis (4-hydroxy-coumarin) (dicoumarol) from Janssen Chimica (Beerse, Belgium); 4H-pyran-one and benzoic acid from Aldrich Chemie (Steinheim, Germany).

PDE activity was measured using a method based on the procedure described by Butcher,[12] which utilizes the 5′-nucleotidase of *Crotalus atrox* venom as a means for hydrolyzing (equation (2)) the phosphodiesterase reaction (equation (1)) product, 5′-AMP, with the subsequent measurement of phosphate (P_i).

$$\text{cAMP} + \text{H}_2\text{O} \rightleftarrows 5'\text{-AMP} \tag{1}$$

$$5'\text{-AMP} + \text{H}_2\text{O} \rightleftarrows \text{Adenosine} + P_i \tag{2}$$

The release of P_i was quantified by the method of Fiske and SubbaRow.[13] All measurements were made at 30°C and pH 7.5.

The assay medium used for the first reaction (equation (1)) was as follows: 0.30 mL cAMP ($1.2 \cdot 10^{-3}$ M) in Tris HCl buffer pH 7.4, 0.15 mL $MgSO_4$ ($1.2 \cdot 10^{-3}$ M) in Tris HCl buffer pH 7.4, 0.15 mL Tris HCl buffer pH 7.4, 0.30 mL PDE (2 or $4 \cdot 10^{-2}$ U/mL) in saline solution (0.9%) or 0.15 mL of compound ($0.8 \cdot 10^{-9}$ M) and 0.15 mL of PDE ($8 \cdot 10^{-2}$ U/mL).

For the second reaction (equation (2)), the mixture was incubated for 30 min at 30°C, adding 0.1 mL of 5′-nucleotidase (0.1%) after the first 20 min. After mixing with trichloroacetic acid (20%), samples were centrifuged. Finally, blank and test samples were prepared to carry out spectroscopic measurements at 660 nm (A_{660}), using calibration curves to convert A_{660} to P_i concentrations. For this, the ε value, previously calculated, was used. ($\varepsilon_{660\text{nm}} = 1.2 \cdot 10^{-4}\ \text{M}^{-1}\ \text{cm}^{-1}$).

The inhibition of PDE activity by DSCG and dicoumarol was determined by a Lineweaver-Burk plot (Figure 1) using different substrate concentrations, from 0.2 to $10 \cdot 10^{-3}$ M. K_m and $V_{\max}$ values were calculated from the straight line without inhibitor. At the origin of coordinates $1/[S]$ became zero and the Lineweaver-Burk equation was transformed into equation (3);

$$\frac{1}{v_o} = \left(1 + \frac{[I]}{K_I}\right) \frac{1}{V_{\max}} \tag{3}$$

where $\frac{1}{v_o}$ is the ordinate value in the straight line with inhibitor when $1/[S] = 0$ and $[I]$ the inhibitor concentration, $4 \cdot 10^{-10}$ M from which K_I values were obtained.

RESULTS AND DISCUSSION

As can be seen from Table 1, DSCG and dicoumarol produce an inhibition of 15–30%, depending on protein concentration. Subsequent kinetic studies showed that cromoglycate and dicoumarol inhibited significantly phosphodiesterase activity, having K_I values, $0.8 \cdot 10^{-9}$ M and $1.6 \cdot 10^{-9}$ M, respectively.

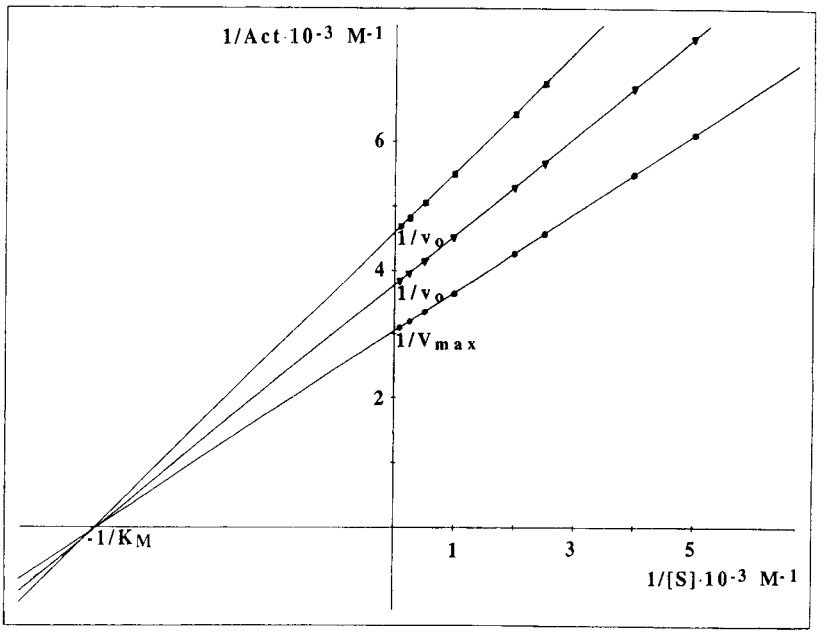
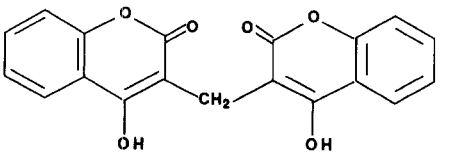
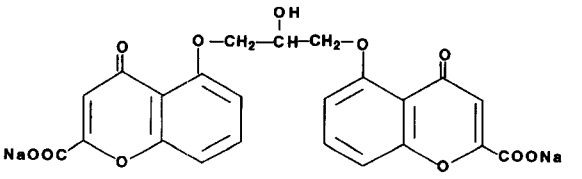


FIGURE 1 Lineweaver-Burk plot of bovine heart cAMP-dependent phosphodiesterase ($4 \cdot 10^{-2}$ U/mL): (●) in the absence of inhibitor, (■) in the presence of sodium cromoglycate (DSCG) ($4 \cdot 10^{-10}$ M) and (▼) in the presence of dicoumarol ($4 \cdot 10^{-10}$ M).



Dicoumarol



Disodium cromoglycate

FIGURE 2

TABLE 1
Effect of disodium cromoglycate (DSCG), dicoumarol (DICUM), coumarin (CUM), 4-pyranone (4-PYR) and benzoic acid (BENZ) on the enzymatic activity of phosphodiesterase (PDE).

System	Drug (M)	Activity Measurement		% Inhibition
		A_{660}	(P_i) (M)	
PDE[a]	—	0.025	$0.21 \cdot 10^{-3}$	—
PDE[b]	—	0.023	$0.19 \cdot 10^{-3}$	—
PDE[a]-DSCG	$4 \cdot 10^{-10}$	0.0175	$0.14 \cdot 10^{-3}$	33.33
PDE[b]-DSCG	$2 \cdot 10^{-10}$	0.019	$0.16 \cdot 10^{-3}$	15.79
PDE[a]-DICUM	$4 \cdot 10^{-10}$	0.020	$0.17 \cdot 10^{-3}$	19.05
PDE[a]-CUM	$4 \cdot 10^{-10}$	0.024	$0.20 \cdot 10^{-3}$	4.76
PDE[a]-PIR	$4 \cdot 10^{-10}$	0.0265	$0.22 \cdot 10^{-3}$	—
PDE[a]-BENZ	$4 \cdot 10^{-10}$	0.023	$0.19 \cdot 10^{-3}$	9.52

$[PDE]^a = 4 \cdot 10^{-2}$ U/mL. $[PDE]^b = 2 \cdot 10^{-2}$ U/mL.

Benzoic acid weakly inhibits PDE activity (<10%) while pyranone is not an inhibitor. As an acidic group is present in the DSCG molecule but not in dicoumarol, (Figure 2) the greater inhibition of phosphodiesterase activity by DSCG may be due to a COOH-protein interaction.

The two dichromones examined inhibit the enzymatic activity of phosphodiesterase. However only DSCG has antiallergic activity. This suggests that, inhibition of phosphodiesterase activity by dicoumarol might not be sufficiently strong to prevent an allergic reaction. Another reason could be that PDE inhibition is not a major involvement in the mode of action of DSCG. To support this latter suggestion, we have observed experimentally that cromoglycate inhibits alkaline phosphatase (ALP) activity.[14] This enzyme is also involved in mast cell secretion being inhibited by DSCG but not by dicoumarol. Consequently, it is possible that the antiallergic effect of cromoglycate may be more related to the inhibition of ALP, than to the inhibition of PDE.

Acknowledgements

We wish to thank The Basque Government and the University of Basque Country for economic and technical support through its programme for training of researchers.

References

1. Metzger, H., Alcaraz, G., Hohman, R., Kinet, J.P., Pribluba, V. and Quarto, R. (1986) *Ann. Rev. Immunol.*, **4**, 419.
2. Cox, J.S.G., Beach, J.E., Blair, A.M.J.N., Clarke, A.J., King, J., Lee, T.B., Loveday, D.E.E., Moss, J.F., Orr, T.S.C. and Richie, J.T. (1970) *Adv. Drug*, **5**, 115.
3. Foreman, J.C., Hallet, M.B. and Mongar, J.L. (1977) *Br. J. Pharmacol.*, **59**, 473P.
4. Sorkin, E.M. and Ward, A. (1986) *Drugs*, **31**, 131.
5. Kuzemko, J.A. (1989) *Respir. Med.*, **83**, (Suppl.), 11.
6. Patalamo, F. and Ruggieri, F. (1989) *Eur. Respir. J.*, (Suppl.), **6**, 556.

7. Juji, F. and Suko, M. (1994) *Ann. Allergy*, **72**(5), 452.
8. Foreman, J.C. and Garland, L.G.J. (1976) *Brit. Med.*, **1**, 820.
9. Wells, E. and Mann, J. (1983) *Biochem. Pharmacol.*, **32**, 837.
10. Foreman, J.C. (1981) *Ann. Rev. Pharmacol. Toxicol.*, **21**, 63.
11. Riley, P.A., Stevens, M.T., Mather, M.E. and Eady, R.P. (1987) *Eur. J. Pharmacol.*, **141**, 143.
12. Butcher, R.W. (1974) In *Methods on Enzymology* (Hardman, J.G. and O'Malley, B.W. (eds.)). Vol. 38, pp. 218–223. Academic Press, New York.
13. Fiske, C.H. and SubbaRow, Y.J. (1925) *Biol. Chem.*, **66**, 375.
14. Ochoa de Aspuru, E. and Zatón, A. (1994) *J. Enz. Inhib.*, **8**, 87.