AFFINITY OF CALCIUM IONS TO THE ANTI-ALLERGIC DRUG, DICROMOGLYCATE

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1. Introduction

1,3-Bis (2-carboxycromon 5-yloxy)-2-hydroxypropane disodium salt (disodium cromoglycate, DSCG) is a widely used drug in the prophylaxis and treatment of IgE-dependent bronchial asthma [1,2]. Accumulated evidence indicates that DSCG inhibits the liberation of the mediators of immediate type allergic reactions initiated by reaginic antibody–antigen interaction. It is not an antagonist of histamine, bradykinin, or the slow-reacting substance of anaphylaxis (SRS-A), nor is it anti-inflammatory [3]. It does however inhibit the degranulation of mast cells and the mediators release in response to various immunologic and non-immunologic stimuli [4–6] and prevents homologous passive cutaneous anaphylactic (PCA) reactions in the rat [7,8]. Both histamine release from isolated rat mast cells, and its inhibition by DSCG were found to be calcium dependent [9,10]. Furthermore, it has been shown that DSCG produces a dose-related inhibition of antigen-stimulated $^{45}Ca^{2+}$ uptake by mast cells [11]. Therefore the suggestion was made that DSCG interferes with the calcium transport across the mast cell membrane thereby inhibiting the anaphylactic release of mediators [12]. Mg^{2+} were found to competitively suppress the effect of calcium [13], whereas the heavier alkaline earth ions, strontium and barium, were both found to be effective in the activation of anaphylactic histamine release yet at higher concentrations than those required with Ca^{2+} [13]. Since the structure of the cromoglycate molecule contains carboxylate, ketonic, aromatic and aliphatic ether groups properly spaced [14] it should be capable of chelating alkaline earth and transition metal ions. This observation taken together with the above-mentioned role of this drug in affecting Ca^{2+} influx and histamine secretion from mast cells prompted us to investigate the direct binding of cromoglycate to alkaline earth and transition metal ions. We have indeed found that cromoglycate binds alkaline earth ions in aqueous and methanolic solutions. In the latter solvent the following affinities were determined by monitoring the fluorescence of the cromoglycate: Mg^{2+}, 1×10^4 M^{-1}; Ca^{2+}, 1×10^5 M^{-1}; Sr^{2+}, 3×10^5 M^{-1}; Ba^{2+}, 1.8×10^6 M^{-1}. The stoichiometry of all complexes is 1:1. The binding characteristics have also been confirmed by NMR measurements, which suggest that the metal ions are coordinated to the two carboxylates of one cromoglycate molecule. This type of chelate leaves several coordination sites on the metal available for interaction with the target site on the mast cell. Thus the drug might putatively act by forming a specific ternary complex with alkaline earth ions and the Ca^{2+} gate on the cell membrane, blocking the influx of these ions induced upon antigen binding to the IgE antibodies on the cell.

2. Materials and methods

DSCG was a kind gift of Fisons Pharmaceut. All chemicals were of analytical grade and were used without further purification. Fluorescence spectra of cromoglycate and its complexes were measured at saturation of the cromoglycate (1.0×10^{-5} M methanolic solutions) with the respective alkaline earth salt (5.0×10^{-3} M). Spectra were recorded in the ratio mode on a Perkin-Elmer MPF 44A spectrofluorometer in a thermostated quartz cuvette (7×7 mm) at 25°C excited at 326 ± 1 mm. The K_a values of cromoglycate with various alkaline earth and transition metal ions were determined by automatic continuous fluorometric

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titrations of a 1×10^{-5} M methanolic DSCG solution with a concentrated methanolic solution of the alkaline earth salt (5×10^{-3} M) on a Perkin-Elmer MPF-44A spectrofluorometer operated in the ratio mode. The intrinsic fluorescence of cromoglycate (emitted at 450 ± 5 nm and excited at 326 ± 1 nm) was enhanced upon binding the metal ions. The titrant was added continuously from a motor-driven Hamilton syringe to the thermostated quartz cuvette (7 × 7 mm) containing the mechanically stirred cromoglycate solution. The fluorescence analog signal was digitized by a transient recorder (Biomation 802, Cupertino, CA.). A minimization computer program based on the Powell algorithm [16] was then applied to the experimental data of the saturation curve. NMR measurements of DSCG in CD_3OD and in $(CD_3)_2SO$ were performed at room temperature on a Bruker 270 MHz spectrometer.

3. Results and discussion

The interactions between the metal ions and cromoglycate were monitored via the intrinsic fluorescence and NMR of the ligand. The ultraviolet spectrum of DSCG in water shows maxima at 320 nm and 260 nm with ϵ_M 1.0×10^4 and 1.8×10^4 ($M^{-1} \cdot cm^{-1}$), respectively. The emission intensity of an aqueous solution of the DSCG salt is quite low (quantum yield: $\phi = 0.004$) but when dissolved in methanol it is enhanced ~2-fold. Addition of Ca^{2+} (as the perchlorate salt), or the other alkaline earth ions, to the methanolic solutions of DSCG causes an enhancement of the emission which reaches a max. 2-fold increase at saturation. The quantum yield of the Ca^{2+} complex is the largest 0.015, and those of Sr^{2+}, Ba^{2+} and Mg^{2+} complexes are lower (0.010, 0.008, 0.006, respectively). The fluorescence spectra of the complexes formed with the latter ions, all excited at 326 nm, are shown in fig.1. There is a blue shift of the emission maximum, relative to that of the sodium salt, which is correlated with the ionic radii of the binding metal ions, with Mg^{2+} being the exception. The relative shifts can be seen in fig.1. A fluorescence titration of DSCG with Ca^{2+} is shown in fig.2 along with the resulting Scatchard plot. The stoichiometry of the Ca^{2+} complex is 1:1, and the binding constant is 1.0×10^5 M^{-1} (25°C). Similar titrations were also carried out with the other alkaline earth ions and with Zn^{2+} and Mn^{2+}. The binding constants

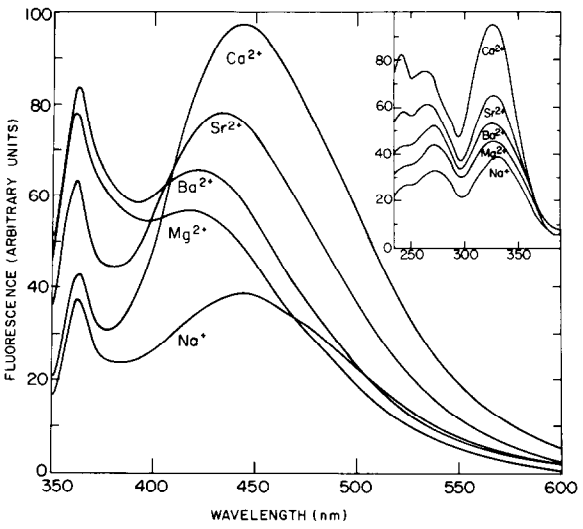

Fig.1. Fluorescence spectra of cromoglycate complexes formed with alkaline earth ions at 25°C excited at 326 ± 1 nm. Insert: Excitation spectra of the same solutions (emission at 450 ± 5 nm).

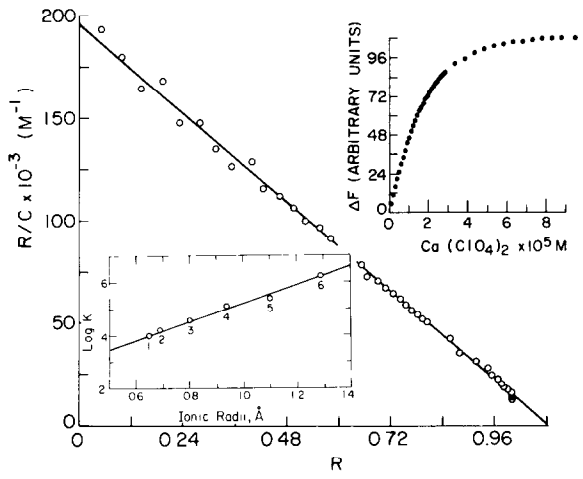

Fig.2. Fluorometric titration of DSCG with $Ca(ClO_4)_2$ in methanol at 25°C. The fluorescence analog signal was digitized by a transient recorder. A minimization computer program based on the Powell algorithm was then applied to the experimental data of the saturation curve (upper insert). The Scatchard plots are the result of the best fit parameters to the experimental data. Lower insert: A linear correlation between the ionic radii of alkaline earth and transition metal ions and their affinity for cromoglycate (expressed as the log of their K_a value). The numbers represent: 1, Mg^{2+}; 2, Zn^{2+}; 3, Mn^{2+}; 4, Ca^{2+}; 5, Sr^{2+}; 6, Ba^{2+}.

for Mg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+} and Mn^{2+} complexes are $1 \times 10^4\ M^{-1}$, $3 \times 10^5\ M^{-1}$, $1.8 \times 10^6\ M^{-1}$, $2.2 \times 10^4\ M^{-1}$, $4.7 \times 10^4\ M^{-1}$, respectively. The stoichiometry for all cases was also found to be 1:1. The relatively low affinity for Mg^{2+} is noteworthy in view of its inhibitory effect on degranulation [13].

NMR measurements of DSCG in $(CD_3)_2SO$, and in CD_3OD show that upon binding of Ca^{2+}, the proton (no. 3) closest to the carboxy-groups undergoes a large downfield chemical shift (0.2 ppm). This could be explained by the cation bound causing a reduction in the electron density at this locus. All the other protons on the ring (no. 6–8) are less affected (0.03 ppm). Thus, one may conclude that the groups chelating the Ca^{2+} are the two carboxylates. Furthermore, the NMR spectrum demonstrates the identity of the protons on the two halves of the bound symmetric ligand molecule. Since the complex is formed in a 1:1 ratio, this implies a structure where both carboxylates of one dicromoglycate molecule are bound to the calcium ion in a symmetrical way.

The inhibition of antigen-induced mediator release from mast cells by cromoglycate has been suggested to be due to its interference with calcium transport across the cell membrane [12]. This still leaves one with the problem of resolving between interference occurring by a direct block of Ca^{2+} gates or indirectly by affecting the cellular level of cAMP [12].

An attractive hypothesis for the mode of action of DSCG is that the Ca^{2+} influx is interfered with by a specific ternary complex formed by the cell membrane gate, a Ca^{2+} and the drug. This hypothesis gains some support from the above-reported affinity between the two latter components and we are presently investigating the binding of the complex between these two to the mast cell membranes.

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