

The effect of sodium cromoglycate on platelets: An in vivo and in vitro approach

José Manuel Tunon-de-Lara, MD,* Patricia Rio, MD,*
Roger Marthan, MD, PhD,** Luce Vuillemin, BSc,***
Dominique Ducassou, MD, PhD,*** and André Taytard, MD*
Pessac and Bordeaux, France

Platelet functions are altered in patients suffering from atopic diseases, including asthma. Such alterations might be related to IgE since platelets bear a specific IgE receptor termed Fc_ε receptor II, and there is some evidence that IgE alone, that is, in the absence of antigen, could modify platelet functions, such as monoamine uptake. Although disodium cromoglycate (DSCG) has been demonstrated to interfere with IgE-mediated activation of human platelets, its effect in the absence of antigen remains unknown. We examined the DSCG effect on IgE-induced alterations both in vivo and in vitro. The effect, in vivo, of a daily intake of 40 mg of DSCG for 4 weeks was assessed on platelet kinetics, studied by a labeling technique on four patients with stable asthma. The effect, in vitro, of DSCG was assessed on IgE-induced alteration in monoamine uptake in platelets from 12 healthy subjects. DSCG significantly increased, in vivo, the mean time constant of platelet-survival curves from 234 ± 40 to 463 ± 150 hours ($p < 0.01$). The amplitude of this effect increased with the patient's serum IgE concentration. DSCG, in vitro, reduced the inhibitory effect of IgE on monoamine uptake by 82%. This effect was concentration dependent and not related to IgE binding. It is concluded that DSCG alters both the survival time of platelets from allergic patients in vivo and the IgE-dependent monoamine uptake in platelets from normal subjects in vitro. These effects are likely to be related to the direct action of DSCG on cells bearing the Fc_ε receptor II and might be independent of the specific trigger. (J ALLERGY CLIN IMMUNOL 1992;89:994-1000.)

Key words: Asthma, IgE, platelet, cromoglycate, monoamine

Previous studies have demonstrated that platelet functions are altered in atopic patients with high concentrations of serum IgE.^{1, 2} The survival time, in vivo, of platelets is shorter in allergic patients with asthma than in healthy subjects.³ In a recent study, we demonstrated that, in vitro, the binding of IgE alone to platelets inhibits monoamine uptake.⁴ Monoamines are taken up by the platelets via an active transport mechanism and may play a role in the control

Abbreviations used

DSCG:	Disodium cromoglycate
ACD:	Acid, citric, dextrose
HKRG:	HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-Krebs-Ringer-glucose medium
¹¹¹ In:	¹¹¹ Indium
Ox:	Oxine
τ:	Time constant
MIBG:	Metaiodobenzylguanidine
FcR:	Fc receptor

From the *Service des Maladies Respiratoires, Hôpital du Haut-Lévêque, Pessac, **Laboratoire de Physiologie, Université de Bordeaux II, Bordeaux, and ***Service de Médecine Nucléaire, Hôpital du Haut-Lévêque, Pessac, France.

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Reprint requests: J. M. Tunon-de-Lara, MD, Service des Maladies Respiratoires, Hôpital du Haut-Lévêque, CHU de Bordeaux, F-33604, Pessac, France.

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of serotonin plasma concentration. Thus, there is a growing body of evidence that IgE is one of the main factors that could alter platelets in asthma, although the actual role of platelets in allergic disorders, namely, in asthma, is still controversial.⁵

DSCG has been demonstrated to inhibit the IgE-mediated activation of human platelets,⁶ since the ef-

fects of DSCG are being directed against IgE/anti-IgE reactions. It is not known, however, whether DSCG has any actions *in vivo* or *in vitro* on platelet function in the presence of IgE alone.

The aim of this work was to analyze the action of DSCG on IgE-induced alterations of human platelets, *in vivo*, by examining platelet kinetics in allergic patients with asthma treated with DSCG, and, *in vitro*, by studying its effect on the IgE-mediated inhibition of platelet monoamine uptake.

MATERIAL AND METHODS

In vivo study of platelet kinetics in allergic patients with asthma

Patients. Since a double study of radiolabeled platelet kinetics before and after the DSCG treatment was necessary, data from only four patients (three women and one man, aged from 20 to 36 years) were finally obtained for this study. All the patients had a history of asthma according to the definition of the American Thoracic Society⁷ and none of the patients were taking any medication except inhaled β -sympathomimetics on an as needed basis. Allergy diagnosis was confirmed by skin testing and measurement of specific IgE in the serum with RAST.

The study was performed during an asymptomatic period, between attacks of asthma. At this time, resting lung function was within normal limits; that is, FEV₁ percent predicted was $93\% \pm 5\%$. Patients' platelet counts were normal ($3.1 \pm 0.5 \times 10^5$ platelets per cubic millimeter), and their serum-IgE concentration was 466 ± 100 UI/ml (normal values, ± 2 SD = 100 ± 50 UI/ml). All patients received 40 mg of DSCG every day for 4 weeks, administered by means of a metered-dose inhaler.

Labeling technique. A total of 40 ml of blood was extracted on ACD A as anticoagulant (citric acid, 1.4 gm; sodium, 2.5 gm; dextrose, 2 gm; completed to 100 ml of H₂O at 9 AM on the day of the study, either before or after administration of DSCG. Blood-cell separation was achieved by slow centrifugation (600 g) for 20 minutes. The platelet-rich plasma was removed and recentrifuged for 10 minutes. The platelet pellet was then resuspended in 1.5 ml of autologous-acidified plasma and labeled during incubation at room temperature for 20 minutes with 1 mCi of ¹¹¹In (Ox 3) (¹¹¹In chloride and ethanol-Ox solution, 1 mg/ml). The platelets were then washed with autologous platelet-poor plasma to eliminate any free indium. The labeling efficiency (ratio of radioactivity in the washed cell pellet to the 1 mCi ¹¹¹In(Ox 3) introduced for the labeling) was approximately 90%. Such a preparation contained <5% white blood cells and 1% red blood cells. The final procedure involved intravenous readministration of the platelets to the subjects 3 hours later.

Measurement of platelet survival. Platelet kinetics were measured 1 day before the patients started receiving DSCG, and then repeated 4 weeks later, according to the recommended methods for radioisotope platelet-survival time studies.⁸

Five minutes after readministration, the length of time necessary for the uniform distribution of the platelets, 2 ml of whole blood was extracted, and the radioactivity was measured with a gamma counter. This measurement served as reference for all the following measurements. Blood samples were taken again 1, 2, 4, 8, 20, 24, 48, and 72 hours after reinjection of the platelets. The percentage of circulating platelets (defined as the ratio of the radioactivity at a given time to that measured at 5 minutes) was then calculated at each of the above times. These percentages versus time were fitted by means of an exponential equation writing: percentage of circulating platelets = $100 \exp^{-t/\tau}$, τ being the time constant; τ was graphically calculated as the time at which the percentage of circulating platelets was 36.8% ($100 \exp^{-1}$).

Determination of platelet transit. To ensure that platelets were not sequestered, we measured their transit time through the lung, liver, and spleen, as previously described.⁹

Transit in the lung was assessed by viewing pulmonary fields by means of a large field gamma camera (type CGR [General Electric CGR USA, Milwaukee, Wis.] acticamera) interfaced to a CGR MAC 7630 computer in a 128 by 128 acquisition matrix. The heart area and the two pulmonary fields were examined via an anterior view. ¹¹¹In radioactivity was measured at equilibrium (at 15 minutes) and at 1, 4, and 20 hours after injection. Correction was made for the physical decay of the radioisotope, and the progression in time of pulmonary radioactivity was plotted on semilogarithmic coordinates. Thus, the pulmonary half-life of the labeled platelets was determined.

Hepatic and splenic transit was assessed by measuring the capture of labeled platelets in each organ at equilibrium (10 minutes after injection, i.e., before the measurement in the lung). Capture was calculated from the ratio of the radioactivity measured in the organ with that measured in the precordial area. The measurement was repeated at each time and compared with the equilibrium radioactivity of each organ after correcting for the physical decay of the radioisotope. A curve was plotted as a function of time in which only the measurement at 48 hours was used to express sequestration; the upper limit of normal sequestration is normally defined as 1.5%.⁹

In vitro studies of monoamine uptake in platelets of subjects with asthma

Subjects. Twelve healthy volunteers, five men and seven women, aged 35 ± 6 years, participated in the study. They did not take any medication, including oral contraceptives. Their platelet count was normal ($2.8 \pm 0.6 \times 10^5$ platelets per cubic millimeter), and their serum-IgE concentration was 37 ± 33 UI/ml.

Blood-platelet isolation. Blood samples were obtained between 8 and 10 AM; 40 ml of venous blood was collected without a tourniquet in a syringe containing 5 ml of ACD anticoagulant. A first centrifugation at 150 g for 25 minutes separated the platelet-rich plasma that was then acidified with 3 ml of ACD. A second centrifugation at 1500 g for 10 minutes allowed the separation of the platelet-poor

plasma from the pellet that was then washed three times in phosphate-buffered saline, pH 6.5, and resuspended in HKRG buffer (HEPES, 25 mmol/L; NaCl, 125 mmol/L; KCl, 4.8 mmol/L; glucose, 5.6 mmol/L; and ascorbic acid, 1 mmol/L) to a final concentration of 10^8 platelets per milliliter.

Immunoglobulins. A solution of monomeric IgE was prepared from monoclonal-purified human IgE (Labiochem, La Jolla, Calif.) and adjusted to a concentration of 1000 IU/ml.

Drugs used. DSCG was a gift from Laboratoires Fisons (Lyon, France). It was suspended in HKRG medium, pH 7.4, to obtain concentrations ranging from 10^{-9} to 10^{-5} mol/L.

MIBG assay. Monoamine uptake was measured by means of a radiolabeled tracer, the ^{125} I-labeled MIBG that is a structural counterpart of guanethidine.

A solution of MIBG (0.2 mg/ml; 10^{-7} mol/L) was prepared to be iodinated with iodine 125 (IK-4 kit, Computerized Imaging Reference Systems Industry, Oris, Gif-Sur-Yvette, France) to obtain a radioactive concentration of 18.5 mBq/ml (0.5 mCi).¹⁰

MIBG uptake was assayed on isolated platelets processed as follows: An initial volume of 250 μ l of platelet suspension was incubated at 37° C under the following different conditions: (1) control, for 90 minutes with HKRG buffer alone; (2) IgE alone, for 45 minutes with HKRG and for 45 minutes with IgE solution, successively; (3) DSCG alone, for 45 minutes with HKRG and for 45 minutes with DSCG solution, successively; (4) DSCG plus IgE, for 45 minutes with DSCG solution and for 45 minutes with IgE solution, successively; and (5) IgE plus DSCG, for 45 minutes with IgE solution and for 45 minutes with DSCG solution, successively.

A volume of 100 μ l of ^{125} I-labeled MIBG was then subsequently added to the initial suspension of platelets (250 μ l) for a final incubation at 37° C. At 10, 30, and 60 minutes, the tubes were rinsed with 2 ml of HKRG and centrifuged at 2000 g for 5 minutes to obtain a pellet, the radioactivity of which was measured with a gamma counter (Packard autogamma 5650, Packard Instrument Co., Downer's Grove, Ill.). Although we previously demonstrated that the duration for the maximal uptake of MIBG is approximately 5 hours,⁴ we analyzed the time course of the MIBG uptake during the first hour, since it corresponds to the highest rate of transport. MIBG uptake was determined as the ratio of the radioactivity measured compared with that of control tubes containing 100 μ l of ^{125} I-labeled MIBG solution. In all experimental conditions, MIBG uptake was normalized to its maximal value (100%) in the control conditions.

IgE binding on platelets. To study the effect of DSCG on IgE binding on platelets, a solution IgE was prepared to be marked with iodine 125 at room temperature to obtain a radioactive concentration of 18.5 mBq/ml (0.5 mCi).¹¹ ^{125} I-labeled IgE binding was then measured with 250 μ l of platelet suspension preincubated 30 minutes at 37° with HKRG or different solutions of DSCG (ranging from 10^{-7} to 10^{-5} mol/L) with a concentration of ^{125} I-labeled IgE ranging from 10 to 10,000 IU/ml.

Statistical analysis. Data are presented as means $\pm$ SD and compared between the different series either with two-tailed Student's *t* test for paired samples, when platelet kinetics were studied, or with two-tailed Student's *t* test for unpaired samples, when monoamine uptake was studied. Differences were considered significant at $p < 0.05$.

RESULTS

In vivo study of platelet kinetics in allergic patients with asthma

As expected, the platelet life span in this group of patients calculated from the extrapolation of the survival curve appeared lower than that previously reported for normal subjects,³ 4.3 ± 0.3 and 8.9 ± 1 days, respectively. The platelet kinetic parameters are summarized in Table I. After the treatment with DSCG, the mean time constant of platelet-survival curves significantly increased from 234 ± 40 to 463 ± 150 hours ($p < 0.01$). Individual platelet-survival curves are illustrated in Fig. 1. For sake of clarity, the percentages of circulating platelets are plotted on a log axis to obtain a linear relationship. Mean data for the patients are illustrated in Fig. 2. The increase in platelet survival depended on patients' serum-IgE concentration (Fig. 2). No difference was observed between the lung images before and after the treatment with DSCG. Hepatic and splenic sequestration of platelets remained in the range of normal values ($<1.5\%$).

In vitro studies of monoamine uptake in platelets of subjects without asthma

As previously reported, MIBG uptake in platelets from the normal subjects increased rapidly until 60 minutes was reached, reaching a value of 0.95 ± 0.01 μ g (100%). A previous incubation with the IgE solution inhibited the MIBG uptake by 66% (Fig. 3, B). This inhibition was significant ($p < 0.05$).

Preincubation with DSCG reduced the inhibitory effect of IgE in a concentration-dependent manner (Fig. 4), whereas DSCG alone had no effect on MIBG uptake (Fig. 3, A and B). However, the effect of DSCG also depended on whether it was applied before or after IgE incubation. For a single concentration of DSCG, 10^{-6} mol/L (i.e., the IC_{90} , the concentration of drug producing 90% of the maximal inhibition), the reversal of the IgE inhibitory effect was only observed when it was applied before IgE incubation. By contrast, when 10^{-6} mol/L of DSCG was applied after IgE incubation, MIBG uptake remained similar to that in the absence of DSCG. In addition, IgE binding to platelets from normal subjects displayed a classic binding curve that was not shifted by a previous incubation with DSCG (Fig. 5).

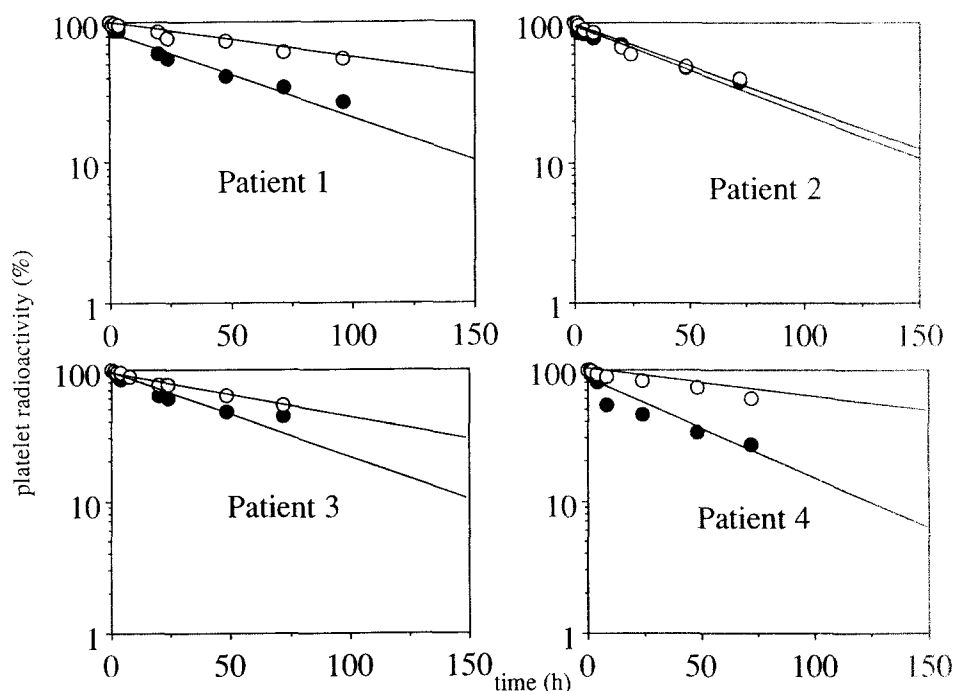


FIG. 1. Individual platelet-survival curves before (●) and after (○) treatment with DSCG; *abscissa*, time in hours; *ordinate*, log axis of platelet radioactivity expressed in percent of its maximal value (100%) measured 5 minutes after readministration of labeled platelets.

TABLE I. Platelet kinetic parameters in patients with asthma before and after a treatment with DSCG

Patient No.	Sex	Age (yr)	Platelets ($n \times 10^9$)	Serum IgE (UI/ml)	Time constant (hr)		Sequestration (%)	
					Before	After	Before	After
1	F	23	303	490	250	600	0.8	1.1
2	F	20	320	200	230	250	0.6	0.9
3	M	36	275	480	275	460	1.1	1.5
4	F	24	344	680	180	540	1.1	1.3
Mean		26	311	463	234	463	0.9	1.2
± SD		3.5	145	99	40	150	0.1	0.1

DISCUSSION

These results reveal that DSCG alters both the survival time of platelets from allergic patients in vivo and the IgE-dependent monoamine uptake in platelets from normal subjects in vitro.

In a previous study, we have demonstrated that platelet kinetics were altered in atopic patients with asthma; that is, the survival time of platelets was shorter in patients with asthma than that in normal subjects and a subpopulation of platelets was affected by an accelerated destruction.³ Since no platelet sequestration was found, we suggested that these results

were related to a functional and/or anatomic lesion of platelets in atopic patients with asthma. In the present study, we demonstrate that DSCG prolongs the survival time of platelets in atopic patients with asthma. Although it has been demonstrated that DSCG inhibits activation of inflammatory cells in vitro,¹² its action on platelets in vivo has not been extensively studied. Nevertheless, it is well-known that inflammatory cells could play a role in the pathophysiology of asthma,¹³ for instance, in the late-phase asthmatic reactions, which are blocked by DSCG.¹⁴ Whether platelets play a large role in this inflammatory cell

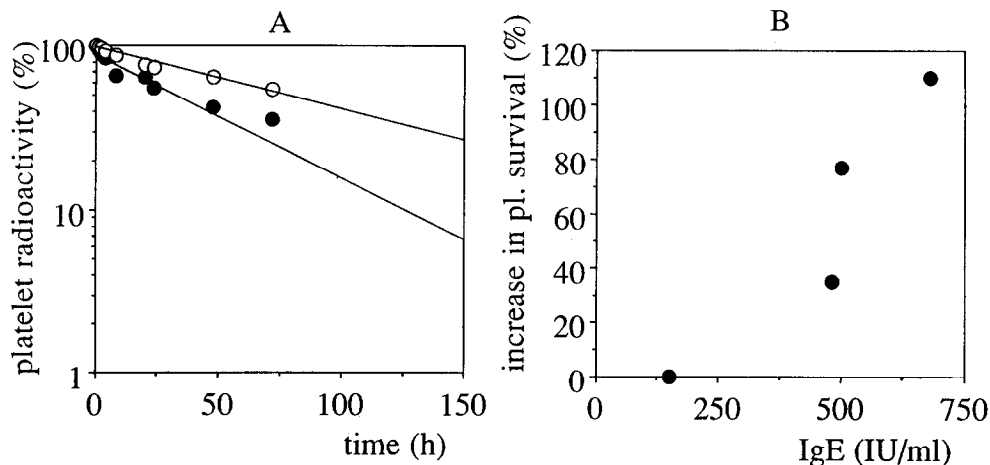


FIG. 2. Mean platelet-survival curve and relationship with individual IgE concentration. **A**, Mean platelet-survival curve before (●) and after (○) treatment with DSCG (same legend as Fig. 1). **B**, Relationship between serum-IgE concentration and DSCG-induced increase in platelet-survival time, calculated as ratio of duration of platelet survival after DSCG to survival before DSCG, expressed in percentage.

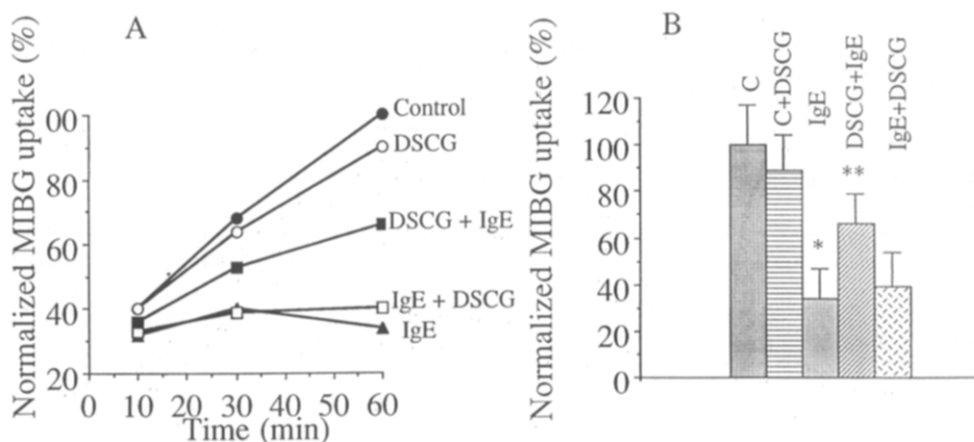


FIG. 3. Uptake of MIBG in platelets. **A**, Time course of MIBG uptake in different incubation conditions; *abscissa*, time in minutes; *ordinate*, percent of MIBG uptake normalized to its maximal value in control conditions (100%); for sake of clarity, SDs are not represented. **B**, Mean values of MIBG uptake at 60 minutes of incubation (* $p < 0.05$ vs control; ** $p < 0.05$ vs IgE). Same ordinate as in A; vertical bars, SD.

response is not well established. However, it is noteworthy that these inflammatory cell types bear the low-affinity receptor for IgE, termed $Fc\epsilon RII$, and that DSCG inhibits their IgE-dependent activation.⁶ In both previous and present studies, the platelet kinetics were altered in patients suffering from allergic asthma with elevated IgE serum concentration. Moreover, the effect of DSCG on platelet-survival time appeared less efficient in patient 2 (Table I) in whom IgE serum concentration was lower than that of the other three

patients. Although studies were done on a small number of patients, we found a significant correlation between serum-IgE concentration and DSCG-induced increase in platelet-survival time. Thus, DSCG action on life span of platelets may depend on IgE-related mechanisms of platelet alteration and may be dependent on the actual level of serum IgE.

IgE-related activation of platelets *in vitro* usually involves IgE bound to the membrane and the specific antigen or anti-IgE that allows the bridging of two

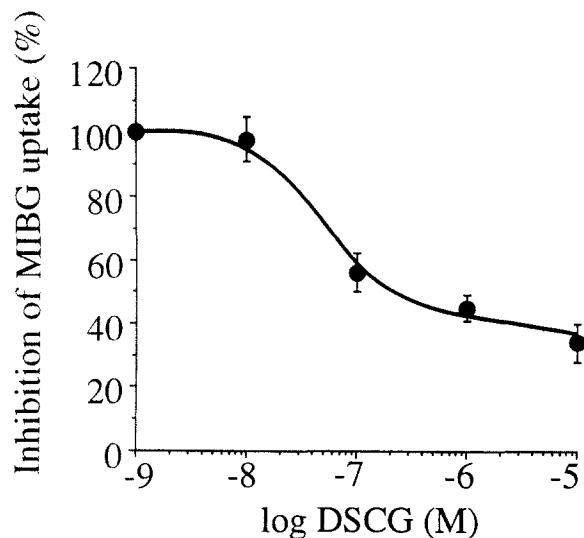


FIG. 4. Concentration-dependent effect of DSCG on IgE-induced inhibition of platelet MIBG uptake; *abscissa*, log axis of DSCG concentration; *ordinate*, percentage of inhibition.

receptors.¹⁵ However, the survival time of platelets is not altered after an allergen-induced bronchospasm.¹⁶ Our results on platelet kinetics may then be explained by an action of IgE other than the action involving the triggering by an antigen or anti-IgE. IgE might have an action on platelets *in vivo* in the absence of the antigen. This hypothesis is supported by some *in vitro* results. In a recent study, we demonstrated that the binding of IgE alone *in vitro* inhibits monoamine uptake in platelets from both allergic patients and normal subjects.⁴ Our present results reveal that DSCG tends to normalize monoamine uptake in spite of the presence of IgE binding to the platelets. This effect of DSCG was specific because it varied in a concentration-dependent manner. Since a preincubation with DSCG solution alone did not alter monoamine uptake, there is some evidence that DSCG interferes with the mechanisms by which IgE inhibits monoamine uptake. Furthermore, DSCG reversed the inhibitory effect of IgE only when it is incubated before the IgE incubation. In addition, the action of DSCG did not depend on the binding of the molecule to the Fc_εR11 since preincubation of platelets with DSCG did not modify the ¹²⁵I-labeled IgE binding.

The mechanism by which DSCG alters the effect of IgE on monoamine uptake remains unknown. The primary mode of action of DSCG is considered to be that of stabilizing mast cells, which prevents the release of mediators.¹⁷ In addition to this effect on cells bearing the high-affinity receptor for IgE (Fc_εR1),

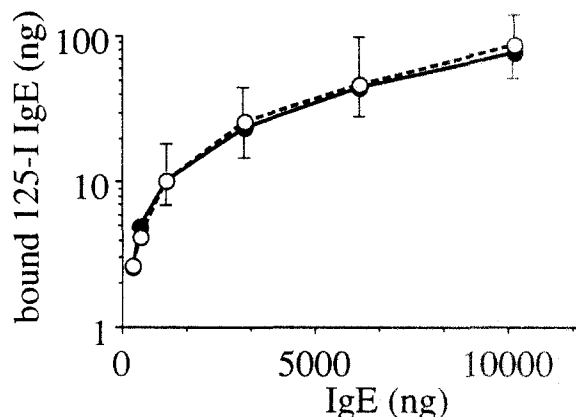


FIG. 5. ¹²⁵I-labeled IgE binding to isolated normal platelets before (●) and after (○) incubation with 10⁻⁶ mol/L of DSCG solution; *abscissa*, total dose of IgE added to medium; *ordinate*, log axis of dose of bound IgE.

DSCG inhibits activation of cells bearing Fc_εR11.^{6, 12} Both these modes of action involve an antibody/antigen mechanism. However, DSCG has been demonstrated to inhibit the *in vivo* bronchoconstriction induced by nonimmunologic triggers, such as exercise,¹⁸ cold air,¹⁹ sulfur dioxide,²⁰ or leukotriene D₄.²¹ The mechanisms involved in these cases are still obscure but may not be associated with an antibody/antigen intervention. One explanation could be "C" fiber sensory nerve, the excitation of which is inhibited by DSCG.²² Another possible explanation is a direct action of the IgE in the absence of antigen, which could modify the cells bearing Fc_εR11. Additional experiments are needed to determine precisely the cellular mechanism of such a direct effect of IgE alone as well as a possible pharmacologic modulation by DSCG.

In summary, we have demonstrated that DSCG prevents both asthma-induced alteration of platelet kinetics *in vivo* and IgE-induced alterations of platelet functions *in vitro*. Whether the effect of DSCG on platelets has any role in its overall effect in controlling asthma requires additional investigations.

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REFERENCES

1. Maccia CA, Gallagher JS, Ataman G, Glueck HI, Brooks SM, Bernstein IL. Platelet thrombopathy in asthmatic patients with elevated immunoglobulin E. *J ALLERGY CLIN IMMUNOL* 1977;59:101-8.
2. Gallagher JS, Bernstein IL, Maccia CA, Splansky GL, Glueck HI. Cyclic platelet dysfunction in IgE-mediated allergy. *J ALLERGY CLIN IMMUNOL* 1978;62:229-35.
3. Taytard A, Guenard H, Vuillemin L, et al. Platelet kinetics in

- stable atopic asthmatic patients. *Am Rev Respir Dis* 1986;134:983-5.
4. Tunon de Lara JM, Vuillemin L, Rondoyanni F, Rio P, Ducassou D, Taytard A. The inhibitory effect of IgE on monoamine uptake in human platelets. *Clin Exp Allergy* 1991;21:109-13.
 5. Capron A, Auriault C, Cesbron JY, et al. Platelets in asthma. In: Kaliner MA, Barnes PJ, Persson CGA, eds. *Asthma: its pathology and treatment*. New York: Marcel-Dekker, 1991: 231-46.
 6. Tsiopoulos A, Lassale P, Joseph M, et al. Effect of disodium cromoglycate on inflammatory cells bearing the Fc epsilon receptor type II. *Int J Immunopharmacol* 1988;10:227-36.
 7. American Thoracic Society. Standard for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-43.
 8. Panel on Diagnostic Application of Radioisotopes in Hematology. International Committee for Standardization in Hematology. Recommended methods for radioisotope platelet survival time studies. *Blood* 1977;50:1137-46.
 9. Van Reenen OR, Lötter MG, Heyns AP, et al. Quantification of the distribution of ¹¹¹In-labeled platelets in organs. *Eur J Nucl Med* 1982;7:80-4.
 10. Guilloteau D, Chalon S, Baulieu JL, et al. Comparison of MIBG and monoamines uptake mechanisms: pharmacological animal and blood platelets studies. *Eur J Nucl Med* 1988;14:341-4.
 11. Fraker PJ, Speck JC. Protein and cellular membrane iodinations with sparingly soluble chloramide 1,3,4,6-tetra chloro-3-6 diphenyl glycoluril. *Biochem Res Commun* 1978;80:849-55.
 12. Kay AB, Walsh GM, Moqbel R, et al. Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. *J ALLERGY CLIN IMMUNOL* 1987;80:1-8.
 13. Laitinen LA, Laitinen A. Pathology of human asthma. In: Kaliner MA, Barnes PJ, Persson CGA, eds. *Asthma: its pathophysiology and treatment*. New York: Marcel-Dekker, 1991:103-34.
 14. Pepys J, Haergraves FE, Chan M, McCarthy DS. Inhibitory effects of disodium cromoglycate on allergen inhalation tests. *Lancet* 1968;2:134-7.
 15. Capron A, Ameisen JC, Joseph M, Auriault C, Tonnel AB, Caen J. New functions for platelets and their pathological implications. *Int Arch Allergy Appl Immunol* 1985;77:107-14.
 16. Hemmendinger S, Pauli G, Tenabene A, et al. Platelet function: aggregation by PAF or sequestration in lung is not modified during immediate or late allergen-induced bronchospasm in man. *J ALLERGY CLIN IMMUNOL* 1989;83:990-6.
 17. Cox JSG. Disodium cromoglycate (FLP 670) (Intal): a specific inhibitor of reaginic antibody/antigen mechanisms. *Nature* 1967;216:1328-9.
 18. Davies SE. Effect of disodium cromoglycate on exercise-induced asthma. *Br Med J* 1968;3:593-9.
 19. Fanta CH, Mc Fadden ER, Ingram RH. Effects of cromolyn sodium on the response to respiratory heat loss in normal subjects. *Am Rev Respir Dis* 1981;123:161-4.
 20. Sheppard D, Nadel JA, Boushey HA. Inhibition of sulfur dioxide-induced bronchoconstriction by sodium cromoglycate in asthmatic subjects. *Am Rev Respir Dis* 1981;124:257-9.
 21. Advenier C, Cerrina J, Duroux P, Floch A, Pradel J, Renier A. Sodium cromoglycate, verapamil and nicardipine antagonism to leukotriene D₄ bronchoconstriction. *Br J Pharmacol* 1983;78:301-6.
 22. Dixon M, Jackson DM, Richards IM. The action of sodium cromoglycate on "C" fibre endings in the dog lung. *Br J Pharmacol* 1980;80:11-3.