The Enhanced Efficacy of Disodium Cromoglycate (DSCG) in DSCG Predosed Rats (37336)

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The exact mode of action of disodium cromoglycate (DSCG) in preventing reagin and nonreagin-induced release of pharmacologic mediators of anaphylaxis has not been definitively established. The drug's inhibition of the Passive Cutaneous Anaphylaxis reaction (PCA) in the rat has been shown (1–4). Various vague references to its possible role in membrane stabilization have been made (2), but no data have been reported which establish the membrane stabilization hypothesis. The biphasic nature of the *in vitro* dose response curve [inhibition can be shown in a dose related fashion up to a concentration of (50–75 μg/ml) while higher concentrations lead to a loss of inhibition] rules out any clear-cut demonstration of a specific site of action (5).

An any rate, the effects of DSCG last far longer than its half-life in the animal (2). The effect of this drug in asthma lasts up to 4.0 hr, when one can show by radioactive labeling of the compound that most, if not all, of the drug is gone in that period of time. This might implicate some enzyme needed for mediator release as the site for DSCG's action.

This paper reports the results of studies in passively sensitized rats that show that pretreatment of rats with DSCG, without antigen, somehow affects the sensitized mast cell site. Thus, inhibition of mediator release by a second treatment with DSCG during antigen challenge can be accomplished at markedly lower concentrations of the drug.

Materials and Methods. Female Sprague–Dawley (Upjohn Co:Upi TUC [SD] Spf) weighing 250 g were skin-sensitized with heat labile rat anti-ovalbumin homocytotropic antibody having a PCA titer of (1:128). After a 72-hr latency period, the animals were challenged with 4 mg ovalbumin (OA) + 5 mg Evans blue dye in 1 ml iv. Thirty minutes later the extravascular blueing that resulted from antigen antibody combination at the skin site was read. Antibody dilutions were used such that in control animals a 4 mm spot is the lowest detectable spot, and 4 or 5 lower dilutions are used to give a range of antibody in each animal. Four to five animals were used for each variable in the experiment. Percent inhibition of the PCA assay was calculated by comparing the spot scores of DSCG treated rats with the spot scores of control rats. The spot score is the total number of detectable spots produced by a series of antibody dilutions divided by the number of animals.

Disodium cromoglycate pretreatment. Passively sensitized rats were injected 2.0 hr before antigen challenge iv with DSCG made up in 0.85% saline at 10, 1.0, and 0.1 mg/kg. No Evans blue or antigen is given at this time. The animals are rested for 2 hr and then controls and DSCG pretreated rats are reinjected iv with 4 mg ovalbumin + 5 mg Evans blue containing a second dose of DSCG at 10, 1.0, and 0.1 mg/kg. After 30 min, the spots are read and compared to controls, and controls without pre-DSCG treatment. DSCG was a gift of Dr. J. S. G. Cox, Fisons Ltd., Loughborough, England.

Results. In order to extend the amount of time needed to manipulate the rat PCA assay, ip injections of DSCG were given and the disappearance of the effect of inhibition of the PCA was measured.

Table I shows that the effect of DSCG does not persist in the animal for extended periods of time. In 25 min most of the

TABLE I. The Time Course of the Effective Half-Life of DSCG in Rats when Given ip Followed by iv Antigen Challenge.[a]

DSCG 10 mg/kg	Time of separation of ip injection and iv challenge	Spot[b] score	% Inhibition
0	—	2.25	—
+	iv at challenge	0	100
+	5 min	1.67	26
+	7	1.67	26
+	10	0.66	73
+	15	1.00	51
+	18	1.25	44
+	20	1.50	33
+	25	1.67	26
+	30	2.0	11
+	60	2.0	11

[a] Animals were challenged with ovalbumin 4 mg + 5 mg Evans blue. Spot score was calculated 30 min later.

[b] Spot score determined from (4) animals per time point.

inhibitory effect of the drug has disappeared in rats. This data confirms the results of others in primates, and rodents showing a short half-life for the drug given ip or iv (2). The serum half-life in man has been shown to be 2 min (2). Since we showed (Table I) that if we waited 2.0 hr in our rat studies after pretreatment with DSCG, this would be sufficient to rule out any drug effect if not residual drug; we used a 2.0-hr delay as a pretreatment time.

Table II shows the "sparing" or "loading" effect of a pretreatment of rats with DSCG on a subsequent concentration of Intal required to cause inhibition when the rat is challenged with antigen. Two points are apparent from this table: (a) It takes less DSCG to achieve inhibition after a DSCG pretreatment (0.1 mg/kg vs 20 mg/kg); therefore, DSCG efficacy has been enhanced, and (b) The pretreatment of the rat with high concentrations of DSCG (20 or 10 mg/kg) prevents the secondary inhibition by 20 or 10 mg/kg DSCG in an unknown fashion (8 vs 75%). This "self-inhibition" has been seen consistently in this assay.

Table III extends the time between the last DSCG injection and antigen challenge time to 20–48 hr to more nearly approximate the mode of DSCG administration in man. In this experiment daily dosing of the rats with 10 mg/kg DSCG for three consecutive days prior to challenge, with 20 hr separating the last DSCG pretreatment from antigen challenge, showed both the sparing and high dose-inhibition effects described above. However, at 48 hr, both effects of sparing and self-inhibition are gone, indicating a finite time period for both of these effects.

TABLE II. The Effect of Previous DSCG Treatment on Subsequent DSCG Inhibition of the Rat (PCA) Assay.

	% Inhibition of PCA				
Primary DSCG treatment, no antigen.	Secondary DSCG treatment with 4 mg OA and 5 mg Evans blue. Concentration mg/kg[c]				
Concentration mg/kg	20	10	1.0	0.1	0
20	8[a]	25	67	58	—
10	58	50	50	33	—
1.0	67	83	41	25	—
0.1	83	91	33	25	—
20 + OA 4 mg + Dye 5 mg[b]	—	—	—	—	75[b]
10 + OA 4 mg + Dye 5 mg[b]	—	—	—	—	63
1.0 + OA 4 mg + Dye 5 mg[b]	—	—	—	—	25
0.1 + OA 4 mg + Dye 5 mg[b]	—	—	—	—	0

[a] This inhibition is the mean percent from (15) animals.

[b] Controls showing that DSCG given with antigen gives inhibition.

[c] Animals rested 2.0 hr between primary iv DSCG injection and iv challenge.

TABLE III. The Effect of Three Daily Treatments of Rats with DSCG on a Subsequent Dose Response of DSCG on the PCA Reaction.

	% Inhibition rat PCA		
Dose response Intal Concentration mg/kg	Control Injected 3× iv with 1.0 ml saline	Predosed Injected 3× iv with 10 mg/kg DSCG[a]	Predosed Injected 3× iv with 10 mg/kg DSCG[c]
10	82[b]	20	83
5.0	47	20	65
1.0	11	58	8
0.1	0	52	0
0.05	ND	47	0

[a] Last dose of DSCG preceded challenge with 4 mg OA + 5 mg Evans blue by 20 hr.

[b] All animals given id injections of rat homocytotropic in 0.1 ml saline 72 hr previous to challenge.

[c] Last dose of DSCG preceded challenge with 4 mg OA + 5 mg Evans blue by 48 hr.

Discussion. The use of DSCG in the rat mast cell assay to prevent histamine release had led to the finding of a biphasic dose–response curve and a time-dependent loss of efficacy of this drug (6). Additionally, there is a dose-dependent inhibition of further, secondary drug effects in this assay (6). This study extends and enlarges these findings in an *in vivo* rat PCA assay to show (a) a very short half-life of the drug, (b) a dose-dependent inhibition of the efficacy of DSCG when given during antigen challenge, and (c) persistence of this effect of the drug for up to 20 hr after treatment.

The usual dose–response curves reported for this drug are meaningless unless one really understands this loading effect or sparing effect on the cell. It is unlikely that any inhibitor with a longer half-life than DSCG is produced, because of lack of detection of any conversion of H^3-DSCG to any other detectable analog (2). One possible explanation may be that some enzyme, needed for mast cell release of histamine, is inactivated and only recovers very slowly. Many of the literature reports showing dose responses with DSCG are over a very narrow range or no dose response is given (1–3, 5, 6).

If one proposes two sites for DSCG's action, (a) one at the cell surface (affected by large concentrations of DSCG by causing less DSCG to enter the cell on a secondary administration), and (b) one in the interior of the cell "at some enzyme location necessary for mediator release," then one might be able to explain the biphasic dose response seen *in vitro* with this drug.

Definitive studies with radioactive DSCG measuring cell-bound drug vs drug in the interior of the cell may lend some insight into the mode of action of this interesting drug.

Summary. The predosing of rats with different doses of DSCG followed by a sufficient time interval so that the drug's effect was gone, then injection of another DSCG dose with antigen led to a high dose inhibition and low dose sparing of the second DSCG dose. The degree of sparing was concentration related and so was the degree of self-inhibition. These effects persisted for a 20-hr dosing regimen but were gone if one gave the last dose 48 hr before challenge.

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