

An antiallergic activity of disodium cromoglycate unrelated to mast cell activation

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Abstract

The pharmacological effects of disodium cromoglycate (DSCG) were studied in rats during the development of reactions to various allergens or carrageenin. DSCG (10 mg/kg and 100 mg/kg, i.v.) showed pronounced inhibitory effects on type I and type III (passive Arthus) allergic reactions. An immunological degranulation of mast cells and a significant decrease in tissue histamine content were observed in type I allergic reactions but not in type III allergic reactions characterized by an apparent infiltration of neutrophils. An antihistaminic agent, promethazine (1 mg/kg, i.v.) was effective only against type I allergic reactions and totally ineffective against type III allergic reactions. Thus, the results obtained above strongly suggest that DSCG exhibits at least two mechanisms of antiallergic action; one is related to mediator release from mast cells and the other is unrelated to mast cell activation.

Introduction

Disodium cromoglycate (DSCG) is known to be a potent inhibitor of the immunological release of chemical mediator(s) secreted from mast cells [1–5] and has been established as an effective therapy in human asthma. A single dose of DSCG blocks the acute obstruction of airways resulting from antigen inhalation [6, 7, 8]. Long-term treatment with DSCG may also alleviate hyperreactivity in airways [9, 10], which would account for the clinical efficacy of DSCG. The asthmatic airway is hypersensitive to various stimuli of immunological reactions and chemical mediators such as histamine and cholinergic agonists, and even cold air, all of which can provoke an asthma attack [11]. The basis for this hyperreactivity is poorly understood, but it is generally accepted that this hyperreactivity in asthmatics may be closely associated with genetic factor(s) [12]. In addition, the continual inflammation of the airway affected by frequent allergic reactions or virus and bacterial infections may

help to promote hyperreactivity [13–16].

It has been widely assumed that DSCG inhibits the mediator-induced bronchial inflammation by inhibiting mast cells and thereby relieves the hyperreactivity. However, little data is available on the effects of DSCG on inflammatory reactions unrelated to mast cell activation. In the present study, we have focused on the antiinflammatory effects of DSCG on rat footpad oedema models and studied the relation between the action of DSCG and mast cell activation.

Materials and methods

Animals

Wistar strain rats were purchased from Shizuoka Agricultural Co-operative Association for Laboratory Animals (Shizuoka, Japan). After holding for at least 7 days, they were used for experiments. JW-NIBS strain rabbits were purchased from the Rabbit Institute (Nishiwaki, Japan) and after about 30 days were used for immunization.

Test compounds

DSCG (Intal, Fisons) and promethazine (Hiberna, Yoshitomi) were dissolved or diluted in saline and administered intravenously.

Antiserum

Rat anti-2, 4-dinitrophenyl-coupled *Ascaris* (DNP-As) antiserum was prepared in Wistar strain female rats weighing 180–200 g as described by Tada and Okumura [17]. One mg of DNP-As was injected into four footpads together with 10^{10} killed *Bordetella pertussis*. Five days later, 0.5 mg of DNP-As was injected intramuscularly into two locations in the back. Eight days after the first immunization, the blood was taken by aortic puncture from the immunized animals and the pooled antiserum was stored at -20°C . The antibody titer of this antiserum was 1:512, as estimated by passive cutaneous anaphylaxis (PCA) in rat (48 hr latent period).

Rabbit antiserum against rat serum or egg albumin (Sigma) was prepared according to the method of Koda et al. [18]. Rabbits weighing 3.0–3.5 kg were immunized with an

equivolume mixture of normal serum taken from male rats weighing 150–200 g or egg albumin in saline (20 mg/ml) and Freund's complete adjuvant (Difco). One ml of the mixture on day 0 (first immunization) and two ml each on days 7, 14 and 21 were injected intramuscularly into both thighs. The antiserum was collected on day 28. Titers of rabbit anti-rat antiserum and rabbit anti-egg albumin antiserum were 1:4000 to 1:8000, respectively, as estimated by PCA tests in the guinea pig (4 hr latent period).

Immediate type footpad swelling

(1) Male rats weighing 140–160 g were used and footpad swelling was elicited in the right hind limb. (2) Rat anti-DNP-As antiserum (0.1 ml) diluted 1:20 with saline was injected subcutaneously into a footpad. After 48 hrs, 0.1 ml of DNP-As in saline (0.5 mg/ml) was injected into the sensitized site. (3) Rabbit anti-rat antiserum was diluted 1:2 with saline and 0.1 ml of the diluted antiserum was injected subcutaneously into a footpad. (4) Rabbit anti-egg albumin antiserum (0.2 ml) was administered intravenously. Two hours later, 0.1 ml of egg albumin in saline (0.25 mg/ml) was injected subcutaneously into a footpad. [5] Carrageenin (Zushi Chemicals) was dissolved in saline and 0.1 ml of 1% carrageenin was injected subcutaneously into a footpad. [6] In a footpad swelling model induced by anti-DNP-As antiserum, test compounds were given intravenously just before elicitation. In other models, they were given twice intravenously, just before and 120 min after initiation of the allergic reaction.

Delayed type footpad swelling

Heat-inactivated *Bacillus Calmette-Guérin* (BCG, Nippon BCG) suspended in phosphate buffered saline (PBS, pH 7.2) was administered intraperitoneally (0.5 mg/0.2 ml/animal) to a male rat weighing 180–200 g. After 7 days, purified protein derivative of tuberculin (PPD, Mitsui) in PBS (0.2 mg/ml) was injected intradermally (0.1 ml/site) into a right hind footpad of the sensitized rat. Test compounds were administered just before and 6 hrs after eliciting the inflammatory response.

Measurement of paw volume

Paw volume was measured with a plethysmometer (Ugo). Swelling was calculated as follows: Paw volume (ml) = volume after eliciting inflammation – volume before eliciting inflammation.

Section for microscopic examination

Representative sections from swollen paws in which inflammation had been induced by anti-DNP-As anti-serum and anti-egg albumin antiserum were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin or toluidine blue for histopathologic examination.

Measurement of histamine content

A reactive site (right footpad) and a normal site (left footpad) were cut off at the heel 24 hrs after initiating inflammation. Each footpad specimen was weighed, chopped into small pieces and suspended in 5 ml of distilled water. Histamine content of the tissue was extracted by boiling for 10 min and determined by the fluorometric method of Shore et al. [19].

Results

Footpad swelling induced by rat anti-DNP-As antiserum

DSCG given just before antigen challenge produced dose-dependent inhibition of this model. Doses of 10 mg/kg and 100 mg/kg produced the most marked inhibitory effects, both comparable to the effects of promethazine (1 mg/kg) (Fig. 1). In order to examine whether

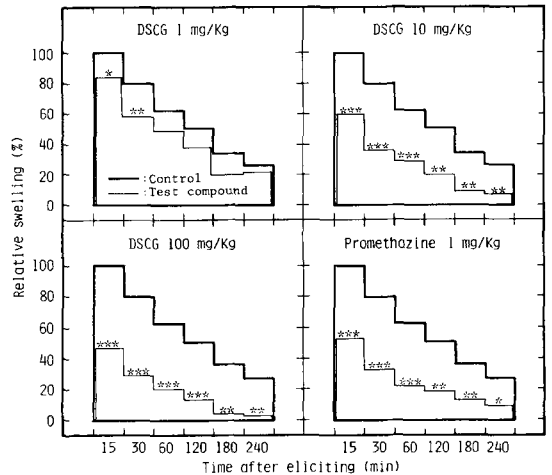


Figure 1

Effects of DSCG and promethazine on allergic footpad swelling in the rat induced by rat anti-DNP-As antiserum. Test compounds were given intravenously just before antigen challenge. 100% swelling was 0.45 ml. Each value indicates a mean of 5 animals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; significant relative to control using Student's *t*-test.

the oedema-inducing activity of anti-DNP-As antiserum was heat-labile, the antiserum was heated at 56°C for 120 min prior to sensitization. Subsequent to this treatment, the swelling at 15 min after antigen challenge was found to be markedly decreased, from 0.45 to 0.08 ml. The histamine content of the swollen footpad (right hind paw) also decreased by about 50% compared with the control footpad (left hind paw) (Table 1). Histopathologic examination revealed a marked degranulation of mast cells (Fig. 2).

Footpad swelling induced by rabbit anti-rat antiserum

DSCG at 100 mg/kg showed a slight but significant inhibitory effect on this response, while at the smaller dose of 10 mg/kg only inhibitory tendency was noted. (Fig. 3). At a dose of 1 mg/kg neither DSCG nor promethazine were effective.

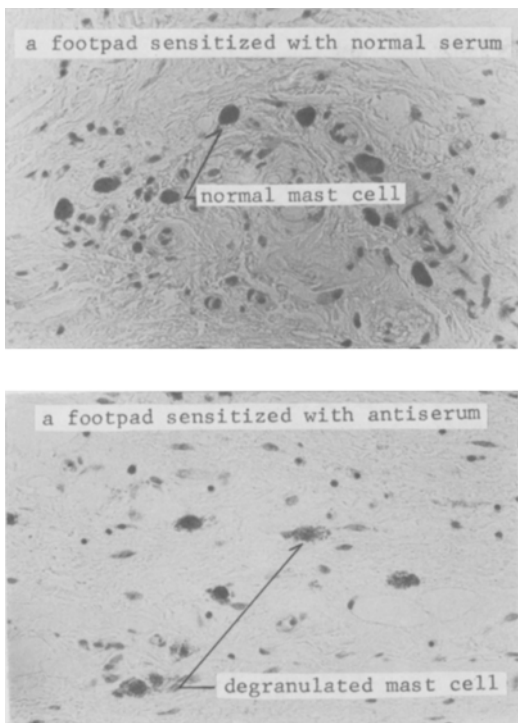


Figure 2
Microscopic findings on the site of an allergic footpad swelling induced by anti-DNP-As antiserum (toluidine blue stain; $\times 120$).

Footpad swelling model induced by rabbit anti-egg albumin antiserum

DSCG was dose-dependently effective in inhibiting this response, the most marked inhibitory effect being observed at 100 mg/kg (Fig. 4). Pro-

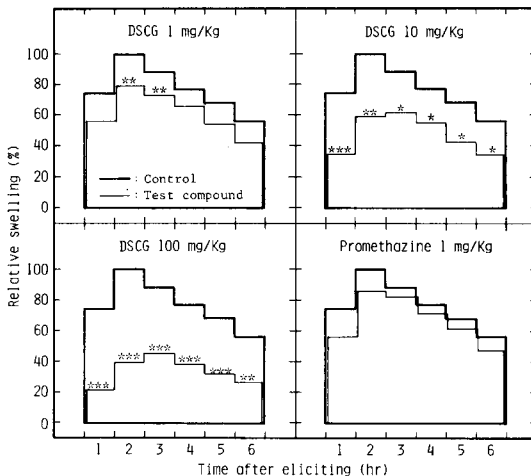


Figure 4
Effects of DSCG and promethazine on allergic footpad swelling in the rat induced by rabbit anti-egg albumin antiserum. 100% swelling was 0.48 ml. Each value indicates a mean of 5 animals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; significant relative to control using Student's t-test.

methazine (1 mg/kg) showed only a tendency towards inhibition, as did the dose of 1 mg/kg of DSCG.

Table I shows that the histamine content of the swollen footpad was nearly equal to that of the control footpad so that no decrease in histamine content after antigen challenge was observed. Microscopic examination revealed infiltration of neutrophils (Fig. 5) but immunolo-

Table 1
Relation between the Allergic Footpad Swelling and the Histamine Content of Footpad.

Serum for sensitization	Relative histamine content v.s. control site (%)
Rat anti-DNP-As antiserum	44.1 \pm 10.34**
Rat normal serum	94.0 \pm 5.44
Rabbit anti-egg albumin antiserum	94.4 \pm 1.96
Rabbit normal serum	92.1 \pm 4.46

Each figure indicates a mean \pm S.E. of 5 animals. A mean histamine content ($\mu\text{g/g}$ tissue) of control footpad was 12.5 in rat anti-DNP-As antiserum, 10.6 in rat normal serum, 19.9 in rabbit anti-egg albumin antiserum or 18.1 in rabbit normal serum. ** $P < 0.01$; significant relative to rat normal serum using Mann-Whitney's U test.

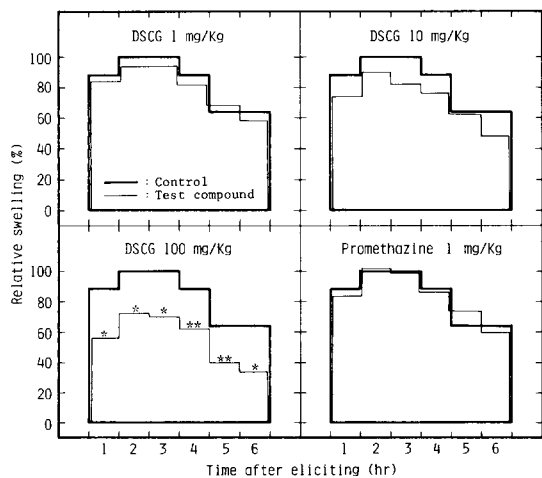


Figure 3
Effects of DSCG and promethazine on allergic footpad swelling in the rat induced by rabbit anti-rat antiserum. 100% swelling was 0.50 ml. Each value indicates a mean of 5 animals. * $P < 0.05$, ** $P < 0.01$; significant relative to control using Student's t-test.

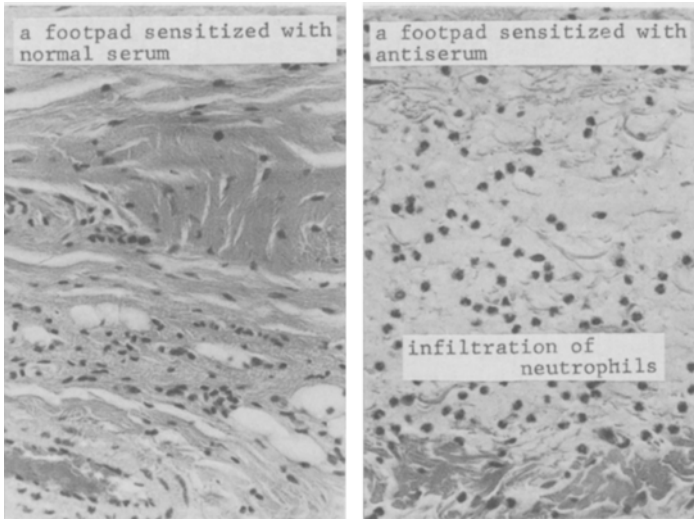


Figure 5
Microscopic findings on the site of an allergic footpad swelling induced by rabbit anti-egg albumin anti-serum (hematoxylin and eosin stain: $\times 120$).

gical degranulation of mast cells was not observed. There appeared to be no definite change in the number of neutrophils in the infiltrate at any dose of DSCG tested, even at the highest dose (100 mg/kg).

Footpad swelling induced by carrageenin

Neither DSCG nor promethazine showed any inhibitory effect in this model (Fig. 6).

Delayed type footpad swelling

Both DSCG and promethazine were totally ineffective in this test (Fig. 7).

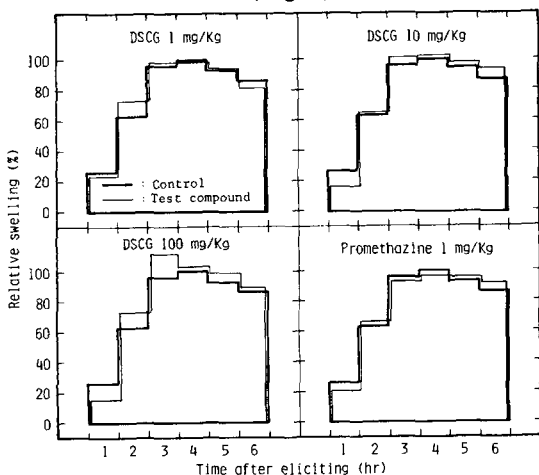


Figure 6
Effects of DSCG and promethazine on footpad swelling in the rat induced by carrageenin. 100% swelling was 0.73 ml. Each value indicates a mean of 5 animals.

Discussion

The anti-allergic effects of DSCG were studied using rat footpad swelling induced by four types of allergic reaction and in response to carrageenin. Intravenous administration of DSCG was markedly effective in inhibiting the two different types of allergic reactions induced by rat anti-DNP-As antiserum on the one hand (Fig. 1) and rabbit anti-egg albumin antiserum on the other (Fig. 4).

The alleviation of the allergic swelling induced by heat-treated anti-DNP-As antiserum (56°C, 120 min) suggests that the oedema was due to an IgE-mediated type I allergic reaction, as classified by Coombs and Gell [20]. Histamine and serotonin, mostly present in mast cells, are

Compound	Swelling volume (ml)			
	0	0,1	0,2	0,3
DSCG 100 mg/Kg				
DSCG 10 mg/Kg				
DSCG 1 mg/Kg				
Promethazine 1 mg/Kg				
Saline				

Figure 7
Effects of DSCG and promethazine on delayed type hypersensitivity in the rat. Swelling was measured 24 hrs after antigen challenge. Each value indicates a mean and S.E. of 5 animals.

known to be the primary chemical mediators of rat type I allergic reactions [21–23] and when released from mast cells cause increased vascular permeability. Indeed, the antihistaminic and antiserotonic agent, promethazine, showed a marked inhibitory effect on the footpad swelling induced by anti-DNP-As anti-serum (Fig. 1). In addition, the decrease in the histamine content of the swollen footpad (Table 1) and the marked degranulation of mast cells at the reactive site (Fig. 2) are characteristic of this type of allergic reaction. These findings confirm that the footpad swelling induced by anti-DNP-As antiserum is a type I allergic reaction elicited by the immunological release of chemical mediator(s) from mast cells. Accordingly, the inhibitory action of DSCG on this type I footpad swelling may be due to the blockade of the immunological release of chemical mediators from mast cells which is a well-established property of the compound [24].

In the allergic footpad swelling induced by rabbit anti-egg albumin antiserum, the marked neutrophil infiltration (Fig. 5) and the maximal swelling at 120 min after antigen challenge (Fig. 4), contrast with the type I allergic reaction in which the maximal swelling was observed after 15 min (Fig. 1). These findings imply that the allergic footpad swelling induced by rabbit anti-egg albumin antiserum is a type III allergic reaction (passive Arthus reaction), as classified by Coombs and Gell [20]. A dose (1 mg/kg) of promethazine which was effective against the type I allergic reaction was found to be totally inactive on this type III footpad swelling (Fig. 4). Neither a decrease in the histamine content of the swollen footpad (Table 1) nor immunological degranulation of mast cells was noted. Therefore, it may be assumed that rat type III allergic footpad swelling may be dependent on some other mechanism(s) than mast cell activation. DSCG showed a marked inhibitory effect on this type III allergic footpad swelling. This strongly suggests that the inhibition by DSCG of rat type III allergic footpad swelling involves an action additional to its major known pharmacological effect as an inhibitor of IgE-mediated immunological release of chemical mediators from mast cells.

It was originally postulated that the clinical efficacy of DSCG might be attributed to its inhibition of immunological release of bronchoactive mediators from mast cells [24]. However, its mode of action in asthmatics remains unclear, since the effects of DSCG on exercise [15, 25, 26]-, SO₂

[15]- or histamine [7, 15, 27]-induced asthma and the alleviation of airway hyperreactivity can not be fully explained by inhibition of IgE-mediated release of chemical mediators. Several attempts have been made to relate the clinical efficacy of DSCG [24, 27–31] to inhibitory actions on α -adrenoceptor [27] and irritant receptors [28] as well as to an inhibitory effect on the bronchial smooth muscle contraction induced by pharmacological stimuli [29], but no previous report is available on the inhibitory effect of DSCG on rat type III allergic footpad swelling as demonstrated in this paper. Our data threw new light on the mode of action of DSCG, because the type III allergic reaction is closely associated with the late asthmatic reaction in man [32, 33] and with viral or bacterial infections which also promote the clinically important symptoms of hyperreactivity [12, 13].

Neutrophils, which phagocytize immunological reactants, are known to play an essential role in type III allergic reaction by releasing lysosomal enzymes, such as cathepsin D and E, which contribute to the development of inflammation [34]. In our rat footpad swelling model, pronounced neutrophil infiltration was observed and a type III allergic reaction was confirmed (Fig. 5). Although any attempt to relate these findings to the mode of action of DSCG are speculative at this time, it is possible that DSCG might block the release of lysosomal enzymes through a membrane stabilizing effect in analogy to its other actions such as the inhibition of mediator release from mast cells [35] and of bronchial smooth muscle contraction [29], as well as α -adrenoceptor antagonism [27]. A previous report from our laboratory demonstrated for the first time that DSCG exhibits a potent stabilizing activity on erythrocyte membranes [30]. Again, the results obtained in the present study strongly suggest that a similar stabilizing effect of DSCG may also be exerted on lysosomal membranes which have some properties in common with erythrocyte membranes [36, 37]. Further experiments on the membrane stabilizing effects of DSCG are in progress in our laboratory.

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