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EFFECT OF DISODIUM CROMOGLYCATE ON ANTIGEN-EVOKED HISTAMINE RELEASE FROM HUMAN SKIN

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SUMMARY

The effect of disodium cromoglycate (DSCG) on antigen-evoked histamine release from IgE-sensitized human skin *in vitro* has been studied using breast skin from six donors. Concentrations of DSCG ranging from 10–200 μM did not produce any consistent effect on histamine release, the results ranging from moderate inhibition to moderate enhancement. With higher concentrations of DSCG (400–500 μM) enhancement of release occurred in nearly all experiments. Variation of antigen concentration did not modify the response to DSCG. These results do not support the possibility that DSCG may be effective in the treatment of immediate hypersensitivity reactions in human skin.

INTRODUCTION

Disodium cromoglycate (DSCG) given by inhalation exerts a protective effect against attacks of bronchospasm provoked by airborne antigens in allergic asthmatic patients (Altounyan, 1967; Pepys *et al.*, 1968). At a cellular level DSCG acts by preventing release of pharmacologically active substances from mast cells and has been shown to inhibit antigen-evoked histamine release from human sensitized lung *in vitro* (Cox, 1967; Sheard & Blair, 1970). In skin, results have been conflicting. DSCG inhibits passive cutaneous anaphylaxis induced by reaginic antibody in the rat (Goose & Blair, 1969) but does not inhibit the Prausnitz–Kustner reaction in human subjects (Assem & Mongar, 1970). *In vitro* DSCG failed to inhibit antigen-evoked histamine release from guinea-pig skin (Tay, Yeoh & Greaves, 1972). We have therefore investigated the effect of DSCG on allergic histamine release from human skin *in vitro*.

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MATERIALS AND METHODS

The method for obtaining histamine release by antigen *in vitro* from human skin sensitized by reaginic (IgE) antibody has been described in detail elsewhere (Greaves, Yamamoto & Fairley, 1972; Yamamoto & Greaves, 1973).

Human skin

Healthy-looking skin removed at mastectomy was used. The subcutaneous fat was trimmed off and either the skin was used fresh or it was stored overnight in an airtight container at 4°C and used within 24 hr of removal.

Human reaginic serum

Reaginic serum was obtained from two donors. One had *Ascaris* hypersensitivity (total serum IgE 930 iu/ml) and the other was from a patient with respiratory allergy to grass pollen (total serum IgE 950 iu/ml). Sera were stored at −20°C and diluted in Tyrode solution prior to use.

Antigen

Ascaris lumbricoides (Bencard Ltd, Brentford, Middlesex) was used in dilution 1:200–1:10,000. A mixed grass pollen antigen (B_2, Bencard Ltd, Brentford, Middlesex 2·5% pollen extract) was used in dilution 1:250.

Preparation of skin slices

Skin was sliced into 500 μm-thick slices using a hand microtome. The histamine content of the slices and spontaneous histamine release from them have been described in a previous paper (Greaves *et al.*, 1972).

Passive sensitization

Slices (three per glass tube) were incubated with 2 ml of a 1:30 dilution of reaginic serum using Tyrode solution as a diluent. The temperature of incubation was 37°C and the duration 2 hr.

Antigen-evoked histamine release

After washing in Tyrode solution the sensitized skin slices were incubated in triplicate for 15 min at 37°C in 4 ml of Tyrode solution containing antigen. The reaction was terminated by decantation of the supernatant. The histamine content of the supernatant and of the residual skin (after boiling in Tyrode and cooling) was determined by bioassay using the atropinized guinea-pig ileum preparation and the percentage of histamine release from sensitized skin by antigen was calculated as described in a previous paper (Greaves *et al.*, 1972). Released histamine was identified as such, using mepyramine as described previously (Greaves *et al.*, 1972). The mean total histamine per sample ranged from 0·15–0·25 μg.

Effect of DSCG on antigen-evoked histamine release

DSCG was obtained from Fisons Ltd Pharmaceutical Division, Loughborough, Leicestershire. Skin samples were pre-incubated in triplicate with either Tyrode or DSCG for 15 min

at 37°C. Incubation was then continued for a further 15 min after adding either Tyrode, (negative control) or antigen. The range of concentrations of DSCG used (10–500 μM) did not affect the response of the isolated guinea-pig ileum preparation to histamine during bioassay.

RESULTS

The results are summarized in Table 1. The effects of concentrations of DSCG ranging from 10–500 μM were studied using skin from six donors. In some experiments the concentration of antigen was varied in order to exclude the possibility that antigen concentration

TABLE 1. Effect of DSCG on antigen-evoked histamine release from human skin

Donor	Antigen* dilution	Antigen-evoked histamine release (%) Concentration of DSCG (μM)					
		0	10	100	200	400	500
1	1:200	17·0	—	—	12·5	—	—
1	1:5000	13·9	—	—	11·5	—	—
2	1:200	29·7	—	—	—	25·7	—
2	1:10,000	20·2	—	—	—	22·5	—
3	1:200	18·1	—	19·9	—	20·3	—
3	1:5000	17·0	—	14·9	—	18·3	—
4†	1:250	18·5	—	15·2	—	—	21·5
5‡	1:250	11·2	—	16·7	—	—	16·0
6	1:250	19·9	19·4	23·6	—	—	—

* Antigen for donors 1–3 was *Ascaris*; antigen for remainder was B_2 (Bencard).

† DSCG added simultaneously with antigen (no pre-incubation).

Negative control (spontaneous) histamine release was zero in all experiments except one (donor 1). In this experiment the Tyrode and DSCG negative controls gave mean releases of 4·5% and 3·9% respectively. The values for antigen-evoked histamine release for donor 1 are given after subtraction of these negative control releases.

influences the effect of DSCG on histamine release. With lower concentrations of DSCG no consistent effect was seen. DSCG (100–200 μM) produced enhancement in two experiments and inhibition in two. In the fifth an enhancement of release in the presence of a 1:200 dilution of antigen was converted to an inhibition in the presence of 1:5000 dilution of antigen. In contrast, the response to higher concentrations (400–500 μM) was more uniform, enhancement occurring in three out of four experiments. In the fourth experiment a dual effect was seen, inhibition of 13·5% occurring with a 1:200 dilution of *Ascaris* antigen, which was converted to an enhancement of 11·4% when the antigen dilution was increased to 1:10,000. Use of lower concentrations of antigen did not produce a consistent effect on response to DSCG.

DISCUSSION

The present results indicate that DSCG does not consistently inhibit antigen-evoked histamine release from human skin passively sensitized by specific IgE antibody *in vitro*.

The efficacy of DSCG in the treatment of allergic (extrinsic) asthma is now well established. *In vitro* evidence suggests that DSCG inhibits the liberation of pharmacological agents from mast cells during immediate hypersensitivity reactions. It does not interfere with antigen–antibody combination, or with the responses of target organs to the agents (Cox, 1967; Assem & Mongar 1970). However, the effectiveness of DSCG as an inhibitor of immediate hypersensitivity reaction depends on the tissue studied. Inhibition of reagin-mediated histamine release from human lung *in vitro* by DSCG has been established in a number of laboratories (Cox, 1967; Sheard & Blair, 1970; Assem & Mongar, 1970). On the other hand Assem and Mongar were unable to demonstrate inhibition of reagin-mediated histamine release from human leucocytes by DSCG. Little work has previously been done on the effect of DSCG on immediate hypersensitivity in skin. Tay *et al.* (1972) were unable to demonstrate inhibition of antigen-evoked histamine release from the skin of actively sensitized guinea-pigs *in vitro* by DSCG, but this may have been because the antibody involved in this reaction is not a reagin. In the rat DSCG inhibits the passive cutaneous anaphylactic reaction mediated by homocytotropic antibody (Goose & Blair, 1969). The present work is in agreement with the finding that DSCG enhanced the P–K reaction (Assem & Mongar, 1970). The results of the *in vivo* and *in vitro* studies taken together make it unlikely that DSCG will prove a useful clinical inhibitor of immediate hypersensitivity in human skin.

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