INHIBITION OF HOMOLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) BY DISODIUM CROMOGLYCATE-RELATED COMPOUNDS

Akihide KODA, Kenichi SAKAMOTO and Yukiyoshi YANAGIHARA
Department of Pharmacology, Gifu College of Pharmacy, Gifu 502, Japan
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Abstract—A study was conducted to examine the effect on homologous PCA in rats of 14 different, newly synthesized compounds, given orally, in relation to the chemical structure of DSCG, when given orally on homologous PCA in rats. The structure-activity relationship of these compounds was also discussed. Among the 7 compounds of the glycerol bischromononyl ether derivatives, 1,3-bis (2-phenyl-4-chromenon-5-yl) oxopropane-2-ol (compound 1) showed the most potent inhibitory activity on PCA. The other compounds showed a moderate or no effect. Two compounds of glycerol bisphenyl ether derivatives had little effect. On the other hand, 1,3-bis (2-carboxy-3-hydroxyphenyl)oxopropane (compound 11) out of alkandiol bisphenyl ether derivatives significantly inhibited PCA, and the other 4 compounds showed a tendency toward inhibition of the reaction. The late inhibition, seen in the biphasic inhibitory pattern induced by compound 11, may be caused by a certain active substance transformed in the body.

We have already reported a comparative study on the anti-allergic effects of disodium baicalein 6-phosphate (BPS) as a monochromone derivative, and of disodium cromoglycate (DSCG) as a dichromone derivative. BPS inhibited not only reaginic (IgE) antibody-mediated reactions, including antigen-induced mediator release from monkey lung, homologous passive cutaneous anaphylaxis (PCA) in rats, and IgE antibody-mediated degranulation of mast cells, but also non-reaginic antibody-mediated reactions such as mediator release from both guinea pig lung sensitized with egg albumin and human lung caused by anti-IgE. Conversely, DSCG showed characteristic properties as an inhibitor of IgE antibody-mediated reaction. From these findings, it was suggested that the functional site of IgE antibody appears to interact with DSCG, with a definite distance between the two chromone-nuclei; while the site of the non-reaginic antibody, IgG, is shorter or longer than that of IgE antibody and is readily fixed with two molecules of BPS (1).

Meanwhile, the development of DSCG contributed greatly to the therapy for asthma, particulary atopic type asthma (2). The drug, however, is ineffective when given orally, as it is poorly absorbed from the gastrointestinal tract. Such being the case, drugs with a mechanism similar to that of DSCG and are which effective when given orally are most feasible for treatment of patients with asthma, particularly children. Several reports on such drugs have been made (1, 3–9).

The present paper describes the effect of oral ingestion of newly synthesized compounds in relation to the chemical structure of DSCG on homologous PCA in rats using their IgE
antibody, and the structure-activity relationship of drugs regarding the inhibition of IgE of antibody-mediated hypersensitivity reaction is discussed.

MATERIALS AND METHODS

Agents: Agents used were the 14 compounds shown in Table 1: 7 compounds of glycerol bischromenonyl ether derivatives (group A); 2 compounds of glycerol bisphenyl ether derivatives (group B); and 5 compounds of alkandiol bisphenyl derivatives (group C). Before use, compounds 9 and 12 were dissolved in physiologic saline containing 0.05 N sodium hydroxide; and compounds 8, 10, 11, 13 and 14 in physiologic saline containing 1% sodium bicarbonate to make a 2% concentration, respectively. pH values of these solutions did not exceed 10. The remaining compounds were suspended in physiologic saline by adding 1/10 amount of carboxymethyl cellulose (CMC) to obtain a similar concentration. N(3',4'-dimethoxyccinnamoyl) anthranilic acid (N-5') was provided by Kissei Pharmaceutical Co. Ltd.. The agent was suspended in physiologic saline, in a similar manner.

Animals: To prepare the antiserum, female Wistar rats weighing about 200 g were used. Male Wistar rats weighing about 150 g were used for the PCA study.

Antiserum: According to the method of Tada and Okumura (10, 11), rats which had been splenectomized 3 to 5 days before the experiment were immunized with 2,4-dinitrophenyl-coupled ascaris extract (DNP-As) mixed with Killed Bordetella pertussis. The homocytotropic antibody titer of this serum (rat anti-DNP-As serum) was approx. 1:256 as estimated by 48-hr PCA.

Homologous passive cutaneous anaphylaxis (PCA): The antiserum, diluted 20-fold with saline, was given intradermally in a dose of 0.1 ml into 3 sites on the shaved backs of intact rats. A similar dose of saline was injected into the other side. After 48 hr, the animals were given i.v. 1.0 ml of 0.5% Evans blue solution containing 2.0 mg of DNP-As as an antigen. Thirty min later, the animals were exsanguinated and the skins were removed. The amount of extravasated dye resulting from PCA was then estimated colorimetrically after extraction by the method of Harada et al. (12).

Antigen-induced degranulation and histamine release from mast cells in the mesenterium: Mesenterium isolated from intact rats was passively sensitized by incubation with the mixture of 1.0 ml Tyrode's solution and 1.5 ml rat anti-DNP-As serum at 37°C for 1 hr. The mesenterium was then washed several times to remove the excess antiserum, and warmed at 37°C for 20 min in Tyrode's solution before incubation with $10^{-4}$ g/ml DNP-As as an antigen for 20 min. The reaction was stopped by cooling with ice. The mesenterium was separated from the incubation medium, and stained with 0.1% toluidine blue. The number of mast cells degranulated as a result of the antigen-antibody reaction was counted under a microscope. The amount of histamine released into the incubation medium was assayed fluorometrically, according to the method of Shore et al. (13). Agents were added to the incubation medium 20 min prior to treatment with the antigen. For the control, a vehicle only was added.
| Group | No. | Structure | R          | n | Name                                                   |
|-------|-----|-----------|------------|---|--------------------------------------------------------|
| A     | 1   |           |            | - | 1,3-bis(2-phenyl-4-chromenon-5-yl)oxypropane-2-ol      |
|       | 2   |           |            | - | 1,3-bis(2-(4-pyridyl)-4-chromenon-5-yl)oxypropane-2-ol |
|       | 3   |           |            | - | 1,3-bis(2-methyl-4-chromenon-5-yl)oxypropane-2-ol      |
|       | 4   | ![Structure](image) | - | CH₃ | 1,3-bis(2-(2-methyl-4-chromenon-5-yl)oxypropane-2-ol    |
|       | 5   | ![Structure](image) | - | - | 1,3-bis(2-(2-thienyl)-4-chromenon-5-yl)oxypropane-2-ol |
|       | 6   | ![Structure](image) | - | OCH₃ | 1,3-bis(2-(3,4,5-trimethoxyphenyl)-4-chromenon-5-yl)oxypropane-2-ol |
|       | 7   | ![Structure](image) | - | OCH₃ | 1,3-bis(2-(3,4-methylenedioxyphenyl)-4-chromenon-5-yl)oxypropane-2-ol |
| B     | 8   |           | -OH        |   | 1,3-bis(2-carboxy-3-hydroxyphenyl)oxypropane-2-ol      |
|       | 9   |           | -NH₂       |   | 1,3-bis(2-carbamoyl-3-hydroxyphenyl)oxypropane-2-ol    |
| C     | 10  |           | -OH        | 2 | 1,2-bis(2-carboxy-3-hydroxyphenyl)oxyethane            |
|       | 11  |           | -OH        | 3 | 1,3-bis(2-carboxy-3-hydroxyphenyl)oxypropane           |
|       | 12  | ![Structure](image) | -NH₂ | 3 | 1,3-bis(2-carbamoyl-3-hydroxyphenyl)oxypropane         |
|       | 13  |           | -OH        | 4 | 1,4-bis(2-carboxy-3-hydroxyphenyl)oxybutane            |
|       | 14  |           | -OH        | 5 | 1,5-bis(2-carboxy-3-hydroxyphenyl)oxypentane           |
RESULTS

Effects of DSCG and related compounds on homologous PCA in rats were examined. The drugs were given p.o. in a dose of 200 mg/kg 2 hr prior to challenge with antigen. For the control, 10 ml/kg of vehicle (saline or 2% CMC saline) alone was given, in a similar manner. The animals were deprived of food for 24 hr before the drug administration, but water was provided ad libitum. As shown in Table 2, the reaction was significantly inhibited by compounds 1, 6 and 7 of group A, particularly by compound 1 {1,3-bis(2-phenyl-4-chromenon-5-yl)oxypropane-2-ol}. In group B, both compounds had little effect. In group C, a significant inhibition was seen with compound 11 {1,3-bis(2-carboxy-3-hydroxyphenyl)oxypropane}. The others showed a tendency toward inhibition of PCA.

As compound 1 showed the most potent inhibitory activity among group A, 200 mg/kg of the agent was given p.o. to rats in 8 groups, at varying times, prior to challenge with antigen, in order to determine time course of the inhibitory activity. The most potent inhibitory activity was observed at the 1-hr pretreatment; and even the 12-hr pretreatment resulted in approx. 50% inhibition (Fig. 1).

Similar experiments were carried out using 200 mg/kg of compound 11, which possessed the most potent inhibitory activity in group C, and the result was compared with that of the other compounds in groups C and B, since these compounds have a common structure with compound 11 in terms of altering bischromone-type to bis-salicylic acid-type. As shown in Figs. 2-4, the inhibition of PCA by compound 11 was observed to have a biphasic pattern.

| Group | Compound | Amount of dye (μg/site) | % Inhibition |
|-------|----------|-------------------------|--------------|
|       |          | Control (without comp.) | Compound     |              |
| DSCG  | 1        | 5.9±1.46                | 6.0±1.07     |              |
|       | 2        | 5.9±1.46                | 1.1±0.28†    | 81.4         |
|       | 3        | 5.9±1.46                | 3.6±0.54     |              |
|       | 4        | 5.9±1.46                | 8.1±0.98     |              |
|       | 5        | 5.9±1.46                | 6.9±1.00     |              |
|       | 6        | 5.9±1.46                | 2.6±0.30*    | 55.9         |
|       | 7        | 5.9±1.46                | 2.4±0.32*    | 59.3         |
| B     | 8        | 5.4±0.42                | 8.6±1.01     |              |
|       | 9        | 8.4±1.27                | 12.8±1.71    |              |
| C     | 10       | 5.2±0.54                | 3.7±0.83     |              |
|       | 11       | 12.5±1.96               | 5.9±2.14*    | 52.8         |
|       | 12       | 12.5±1.96               | 9.2±2.42     |              |
|       | 13       | 5.1±0.48                | 3.4±0.74     |              |
|       | 14       | 5.1±0.48                | 4.3±0.91     |              |

Administrations were 200 mg/kg p.o. 2 hr prior to challenge. Each value represents the mean±SE of 5 animals. *†: Statistical significance from the control at p<0.05 and p<0.01, respectively.
Namely, the reaction was dramatically inhibited by a 30-min pretreatment; diminished by 1-hr and 2-hr pretreatments; and recurred by the pretreatment on and after 4 hr.

**FIG. 1.** Time course of inhibitory activity of compound 1 on homologous PCA in rats. The compound was given 200 mg/kg p.o. at varying times prior to challenge. Each group included 5 animals.

**FIG. 2.** Time course of inhibitory activity of compounds 11, 10, 13 and 14 on homologous PCA in rats. Compounds were given 200 mg/kg p.o. at varying times prior to challenge. Each group included 5 animals.
The inhibition by 12-hr to 24-hr pretreatments was approximately to the same degree as that of the 30-min pretreatment. In contrast to compound 11, the other compounds did

**Fig. 3.** Time course of inhibitory activity of compounds 11 and 12 on homologous PCA in rats. Compounds were given 200 mg/kg p.o. at varying times prior to challenge. Each group included 5 animals.

**Fig. 4.** Time course of inhibitory activity of compounds 11, 8 and 9 on homologous PCA in rats. Compounds were given 200 mg/kg p.o. at varying times prior to challenge. Each group included 5 animals.
not show such a pattern of inhibition and their most potent activities were generally observed
by 1-hr to 2-hr pretreatments, though the activities were fairly weak as compared with that
of compound 11.

The biphasic inhibition (early and late inhibitions) by compound 11 was also found
in the case of i.p. pretreatment (Fig. 5). Since the late inhibition appeared to result from
biotransformation of the agent, the following experiment was conducted for confirmation.

Intact rats in 2 groups were given 200 mg/kg of compound 11 i.p. and serum was collected
at 1 hr and 12 hr after the administration, respectively. The effect of the serum on PCA
is shown in Fig. 6, where 20 ml/kg of serum was given i.p. 2 hr prior to challenge. Sera
collected 1 hr and 12 hr after the drug administration significantly inhibited the PCA, parti-
cularly the serum collected 12 hr after. This result suggests that the serum collected 12 hr
after the drug administration contains a certain active substance transformed in the body
from compound 11.

An in vitro study was then done to confirm the above finding. Rats were exsanguinated
24 hr after deprivation of food and the livers were excised and perfused with 0.1 M Krebs
Ringer phosphate-buffered saline (pH 7.4) to clear of blood and chopped into slices
0.5 mm thick. One gram of the slices was added to 9 ml of the same buffered saline followed

![Fig. 5. Time course of inhibitory activity of compound 11 in homologous PCA in
rats. The compound was given 100 mg/kg i.p. at varying times prior to challenge.
Each group included 5 animals.](image)

![Fig. 6. Effect of serum of rats treated with compound 11 on homologous PCA.
Serum was given 20 ml/kg i.p. 2 hr prior to challenge. a; normal rat serum, b, c;
serum collected at 1 hr and 12 hr after the administration, respectively. Each
group included 5 animals. *: Statistical significance from the control at p<0.05
and p<0.01, respectively.](image)
by the addition of 1 ml of 2% compound 11. Incubation was performed at 37°C for varying times and the mixture was centrifuged 1,000 rpm at 0°C for 15 min. The resultant supernatant was given in a dose of 10 ml/kg i.v. to rats 15 min prior to challenge. In proportion to incubation time the inhibitory activity increased as shown in Table 3.

A similar experiment was conducted using liver microsome of rats. Microsome fraction was prepared as follows: supernatant obtained from 20% liver homogenate in 1.15% potassium chloride through centrifugation of 10,000 rpm at 0°C for 20 min was recentrifuged 30,000 rpm at 0°C for 60 min. The resultant precipitate, equivalent to 1 g of liver, was suspended in 2 ml of Krebs Ringer phosphate-buffered saline (pH 7.4) and used as a microsome fraction. The reaction mixture, composed of 7.5 ml microsome fraction; 150 μmoles glucose-6-phosphate; 45 μmoles niacin adenine dinucleotide phosphate; 1.5 units glucose-6-phosphate dehydrogenase; 0.2% final concentration of compound 11; and the buffered saline to make 15 ml, was incubated at 37°C for varying times. After the mixture was centrifuged to obtain the supernatant, (as was done in the case of the experiment with the liver slices), the supernatant was subjected to the PCA study. The result is shown in Table 4, where the supernatant was given in a dose of 10 ml/kg i.v. to rats 15 min prior to challenge. Compound 11, without treatment of microsomes, in a dose of 20 mg/kg i.v.,

| Group          | Amount of dye (μg/site) (% Inhibition) |
|---------------|---------------------------------------|
|               | Incubation time (hr)                  |
|               | 0.5        | 1          | 2          | 4          | 6          |
| Control       | 15.0±1.37  |            |            |            |            |
| Compound 11   | 9.7±0.46†  | 11.3±2.36  | 10.5±1.43* | 10.8±1.17* | 9.1±0.89†  | 7.3±1.57†  |

Rats were administered 20 mg/kg of compound 11, incubated with liver slice, i.v. 15 min prior to challenge. Each value represents the mean±SE of 4 experiments. Control; medium incubated with liver slice, a; non-treated compound 11. *;†; Statistical significance from the control at p<0.05 and p<0.01, respectively.

| Group          | Amount of dye (μg/site) (% Inhibition) |
|---------------|---------------------------------------|
|               | Incubation time (hr)                  |
|               | 0.5        | 1          | 2          | 4          | 6          |
| Control       | 23.4±1.46  |            |            |            |            |
| Compound 11   | 19.4±3.08* | 19.2±1.07* | 18.8±1.03* | 18.2±1.68* | 18.3±1.75* | 17.8±2.05* |

Rats were administered 20 mg/kg of compound 11, incubated with liver microsome, i.v. 15 min prior to challenge. Each value represents the mean±SE of 4 experiments. Control; medium incubated with liver microsome, a; non-treated compound 11. *; Statistical significance from the control at p<0.05.
showed a tendency toward inhibition of PCA. On the other hand, PCA was significantly inhibited by the agent treated with the liver microsome.

Since the most potent inhibitory activity against the PCA was found in compound 1, the effects of this compound on antigen-induced degranulation and histamine release from mast cells in the mesenterium of the rat were examined. As shown in Table 5, both reactions were significantly inhibited by compound 1 in concentrations of $10^{-5}$ to $10^{-6}$ g/ml, to the same degree as N-5' which was used as a comparative.

**DISCUSSION**

In our search for drugs which when given orally inhibit IgE-mediated hypersensitivity reaction, we studied several newly synthesized compounds related to the chemical structure of DSCG: 7 compounds of glycerol bischromenonyl ether derivatives (group A); 2 compounds of glycerol bisphenyl ether derivatives (group B); and 5 compounds of alkandiol bisphenyl ether derivatives (group C).

In group A, compounds 1, 6 and 7, which substituted respectively phenyl, 2-furyl and 3, 4-methylenedioxophenyl radicals for carboxyl radical in the 2-position of chromone-nuclei in the chemical structure of DSCG, significantly inhibited homologous PCA in rats. Compound 1 was particularly potent and showed 81.4% inhibition of the reaction. The other compounds showed only a tendency toward inhibition of the reaction. The potent inhibitory activity of compound 1 appears to be the result of excellent absorption from the gastrointestinal tract due to the high lipid solubility. This compound showed a potent inhibition of antigen-induced degranulation and histamine release from mast cells, as did N-5' which is a characteristic inhibitor of IgE antibody-mediated hypersensitivity reaction (9, 14).

Compounds 8 and 9 in group B, which possess the form of open-structure of γ-pyrene, had little effect. However compound 11 in group C showed a significant inhibition of PCA and the others showed a tendency to inhibit the reaction. Thus, the inhibition of IgE antibody-mediated reaction may not always depend on bischromone-nuclei but on bis-type
of salicylic acid. Since compound 8, when compared with compound 11, did not show any effects, the hydroxy radical in the 2-position of glycerol may not be essential for evidence of the activity. Compound 12, a substitute of carbamoyl radical for carboxyl radical of compound 11, showed only a slight inhibition, so that the latter radical appears to be essential for exhibition of the activity. In contrast to compound 11, compounds possessing a different number of methylene group (compounds 10, 13 and 14) slightly inhibited the reaction. Thus, compound 11 as a structure of 2 molecules of salicylic acid connecting with three methylene bridge appears to be an excellent inhibitor of IgE-mediated reaction. The present result appears to support the speculation described in a previous paper (1), in which the functional site of IgE highly interacts with the structure of DSCG at a definite distance between the two chromone-nuclei. In order to confirm this possibility, studies are now under way using the other experimental systems mediated by both IgE and IgG antibodies.

It is interesting that time course of the inhibitory activity of compound 11 showed a biphasic pattern not seen with the other compounds. The early inhibition occurred for a short time after the administration, but the late inhibition remained for a fairly long time. This result indicates that compound 11 is easily transformed to a certain active metabolite in the body. This is supported by the evidence that compound 11 given i.v. was mutable, as seen in Tables 3 and 4. It was also shown in the in vitro study that such a metabolism occurred with liver microsomes. We were unable to find an active metabolite because of the small quantity to be isolated. The use of compound 11 labeled with radioactive isotope might allow for such an isolation.

The active metabolite from compound 11, as well as compound 1, should contribute considerably to the therapy for atopic diseases mediated by the IgE antibody, due to inhibition of the second stage of immediate hypersensitivity reaction (15).

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