INHIBITION OF HYPERSENSITIVITY REACTIONS BY SOLUBLE DERIVATIVES OF BAICALEIN

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Accepted September 11, 1975

Abstract—Baicalein, a flavonoid, is anti-allergic but only slightly soluble in water. The soluble derivatives of baicalein, disodium baicalein-6-phosphate (BPS) and sodium baicalein-6-sulfate (BSS), were synthesized and examined regarding their effects on hypersensitivity reactions. These derivatives inhibited type I and II reactions as classified by Coombs and Gell. The Arthus reaction belonging to type III reaction, however, was hardly affected with either BPS or BSS. The experimental asthma caused by passive systemic anaphylaxis in guinea pigs was prevented with application of BPS. Thus even by the oral route, BPS appears to be clinically applicable to extensive allergy related diseases.

Baicalin is a flavone glycoside contained in the radix of Scutellaria baicalensis GEORG (Woogon), which has been used from ancient times in Chinese medicine as a remedy for allergic diathesis and inflammation. Hitherto, the authors (1–3) have investigated the pharmacological actions of baicalin and its aglycone, baicalein, and reported that each showed a potent protective action against anaphylaxis, i.e. systemic and cutaneous anaphylaxis were prevented in guinea pigs and/or mice, and release of histamine and SRS-A from sensitized guinea pig lung treated in vitro was also inhibited. The functional structure in baicalin may depend upon a part of baicalein, since the inhibitory potency of baicalin was much the same as that of baicalein in an equal molar concentration. The most potent inhibition by baicalein was found in anaphylaxis of tracheal muscle. The contractile response of guinea pig tracheal muscle to the antigen-antibody reaction was suppressed dramatically by the pretreatment of baicalein both in vitro and in vivo. In addition, baicalein moderately antagonized anaphylactic mediators in the isolated ileum of guinea pig.

![Structural formulas](image-url)

**Fig. 1.** Structural formulas of baicalein and its soluble derivatives contrasted with that of disodium cromoglycate.
and relaxed the tracheal muscle fairly well. Thus it would appear that baicalein may be useful for treating allergic disorders, in particular asthma. In the present paper, the anti-allergic actions of soluble derivatives of baicalein, disodium baicalein-6-phosphate (BPS) and sodium baicalein-6-sulfate (BSS), were investigated and the structural formulas of these compounds as contrasted with that of disodium cromoglycate (DSCG), a specific inhibitor of reagin-mediated allergic reaction (4) are shown in Fig. 1.

MATERIALS AND METHODS

Animals: Hartley guinea pigs of both sexes weighing 260 to 350 g, male Wistar rats weighing 120 to 180 g, and male Albino rabbits weighing 2 to 3 kg were used.

Drugs: Baicalein, disodium baicalein-6-phosphate (BPS) and sodium baicalein-6-sulfate (BSS) were gifts from Takeda Chemical Industries Ltd, Japan. Aminophylline, isoproterenol sulfate, chlorpheniramine maleate and prednisolone were purchased from Nakarai Co. Ltd, Japan.

Preparation of antisera: Rabbit anti-egg albumin sera (Rab anti-Ea) were prepared from rabbits which had been immunized by injecting 10 mg of egg albumin emulsified in complete Freund’s adjuvant i.m. 4 times weekly. The serum was obtained 7 to 10 days after the last injection and lyophilized until use. Rat anti-dinitrophenylated ascarsis extract sera (Rat anti-DNP-As) were prepared according to the method of Tada and Okumura (5). Rats were immunized by injecting 1 mg of DNP-As mixed with 10^10 killed Bordetella pertussis into the four foot pads. Five days later, the animals were given a booster of 0.5 mg of DNP-As alone into the muscles of the back. Serum collected from each animal 3 to 4 days after the last injection was pooled and frozen until use. The antibody titre of this serum was 1:128 to 1:256 as estimated by 48 hr homologous passive cutaneous anaphylaxis (PCA). Rabbit anti-rat sera (Rab anti-rat) were prepared from rabbits which had been immunized by injecting 1.0 ml of rat serum i.v. every other day for a total of 10 doses. The serum was obtained on the 20th day after the last injection and lyophilized until use. Anti-sheep erythrocyte sera (hemolysin) were prepared from rabbits which had been immunized by injecting 10^9 sheep erythrocytes i.v. every other day for a total of 10 doses. The serum was also obtained on the 10th day after the last injection and frozen until use.

Heterologous PCA: Heterologous PCA in guinea pigs was carried out by the method of Ovary (6). Guinea pigs whose left backs had been shaved one day before were given 0.05 ml of Rab anti-Ea diluted 16 fold with physiological saline i.d. into 3 sites. After 3 hr, 0.05 ml of 0.1% histamine was similarly injected into the other side of the back followed by an i.v. injection of 1.0 ml of 0.5% Evans blue solution containing 5 mg of the antigen. Thirty min later, the animals were sacrificed by exsanguination and their skins removed to measure the blueing spot, the result of PCA. After this dimension was measured, the amount of the dye was estimated colorimetrically according to the method of Harada et al. (7). Per cent inhibition of PCA was calculated using the following formula: \( \frac{Dc-Ds}{Dc} \times 100 \), in which \( Dc \) represents the amount of dye leaked in the control test while \( Ds \) represents the
amount of dye in the test using the substances.

Homologous PCA: Homologous PCA was carried out according to the method of Tada and Okumura (5). Rat anti-DNP-As diluted 20 fold with physiological saline was injected i.d. in 0.1 ml dose into 3 sites on the shaved left backs of normal rats. Into the other side the same dose of physiological saline was similarly injected. After 48 hr, the animals were given 1.0 ml of 0.25% Evans blue solution containing 2 mg of the antigen i.v., and were sacrificed 30 min later. Measurement of the blueing spot was carried out as above.

Reversed cutaneous anaphylaxis (RCA): Measurement was done according to the method of Ungar et al. (8). The lyophilized Rab anti-rat was dissolved in 1% Evans blue to make a 14% solution. This solution was injected i.d. in 0.05 ml dose into 3 sites on the left backs of rats shaved one day before. Into the other side the same dose of physiological saline containing 1% Evans blue was similarly injected. Two hr thereafter, the animals were sacrificed by exsanguination and the skin removed. Samples were taken from the injected regions of the skin using a punch with a diameter of 12 mm after which they were weighed. Inflammation caused by RCA was calculated by the following formula:

\[ I = \frac{W_i - W_s}{W_s} \times 100, \]

in which \( W_i \) represents the weight of the inflamed region while \( W_s \) represents that of the region treated with physiological saline.

Schultz-Dale reaction: Guinea pigs were sensitized with i.p. and i.m. injections of egg albumin in a dose of 20 mg each, and 4 days later were given a booster of each 100 mg of the antigen through the same routes. Four weeks thereafter, the excised ileum and trachea were tested for the Schultz-Dale reaction in Tyrode's solution at 30 °C in the former and 37 °C in the latter respectively.

Arthus reaction: Rabbits were immunized by injecting 10 mg of egg albumin emulsified in complete Freund's adjuvant i.m. 4 times weekly. Ten days after the last injection, 0.2 ml of 1.0% antigen was injected i.d. into 8 sites on the shaved backs of the animals. At 1, 2, 4, 6, 12 and 24 hr after the injection, the dimension of inflamed region was measured macroscopically.

Forssman cutaneous vasculitis: Guinea pigs were injected with 0.1 ml of hemolyzin diluted 8 fold with physiological saline i.d. into their shaved backs followed by an i.v. injection of 1.0 ml of 1% Evans blue. After 1 hr, the animals were sacrificed by exsanguination and the skin was removed. The blueing spot caused by the Forssman cutaneous vasculitis was measured by the same method as was used for PCA.

Experimental asthma: Guinea pigs were sensitized with an intracardiac injection of 1.0 ml of Rab anti-Ea. After 24 hr, the animals were given 1/4 lethal dose of the antigen i.v. to provoke an anaphylactic reaction. The rate and depth of respiration were recorded on a smoked drum by the method described previously (3). The carotid blood pressure was also simultaneously recorded.

RESULTS

Heterologous PCA

The effects of BPS and BSS on heterologous PCA in guinea pigs were compared with
those of certain drugs including aminophylline, isoproterenol, chlorpheniramine and prednisolone in a dose of 5 mg/kg i.v. at 10 min prior to challenge. As shown in Table 1, all of the substances tested, except for aminophylline, showed an inhibition of the dye leakage caused by PCA, with BPS showing the most potent activity, that is a 69.0% inhibition, while about 30% inhibition was seen in the others. An increase in capillary permeability after an i.d. injection of histamine was also inhibited with all of the substances. The inhibitory activities of BPS and BSS, however, were only 17.1% and 14.3% respectively those are fairly weak compared to those of isoproterenol and chlorpheniramine. Thus, the inhibition of PCA by BPS and BSS did not appear to depend upon their antihistaminic activities.

**Homologous PCA**

Regarding homologous PCA, the effect of substances given in a similar manner to those in the case of heterologous PCA is shown in Table 2. The dimension of the dye leakage caused by PCA was inhibited by the substances, with the exceptions of BSS and amino-

| TABLE 1. Heterologous passive cutaneous anaphylaxis and histamine cutaneous response in guinea pigs |
|---------------------------------------------------------------|
| **Substance** | **PCA** | **Histamine cutaneous response** |
| | µg/site | % Inhibition | µg/site | % Inhibition |
| Control | 8.4 ± 1.36 | | 3.5 ± 0.81 | |
| BPS | 2.6 ± 0.53** | 69.0 | 2.9 ± 0.23 | 17.1 |
| BSS | 6.1 ± 0.71 | 27.4 | 3.0 ± 0.25 | 14.3 |
| Aminophylline | 8.0 ± 0.24 | 0 | 2.6 ± 0.27 | 25.7 |
| Isoproterenol | 5.9 ± 0.51 | 30.8 | 2.0 ± 0.22 | 42.9 |
| Chlorpheniramine | 5.4 ± 0.77 | 35.7 | 2.3 ± 0.32 | 34.3 |
| Prednisolone | 5.8 ± 1.39 | 31.0 | 2.9 ± 0.15 | 17.1 |

Administrations were 5 mg/kg i.v. 10 min prior to challenge. Each value indicates the mean ± S.E. of 6 experiments except for 9 experiments with the control. **: Statistical significance from control at p<0.01.

| TABLE 2. Homologous passive cutaneous anaphylaxis in rats |
|---------------------------------------------------------------|
| **Substance** | **Dimension** | **Amount of dye** |
| | mm² | % Inhibition | µg/site | % Inhibition |
| Control | 58.8 ± 1.95 | 5.6 ± 0.33 | |
| BPS | 48.9 ± 2.06** | 18.9 | 3.9 ± 0.39** | 30.2 |
| BSS | 55.9 ± 2.28 | 5.5 ± 0.29 | |
| Aminophylline | 53.6 ± 3.48 | 4.4 ± 0.23 | 21.4 |
| Isoproterenol | 38.4 ± 1.52** | 34.7 | 3.2 ± 0.27** | 42.5 |
| Chlorpheniramine | 44.4 ± 3.26** | 24.5 | 2.3 ± 0.35** | 58.9 |
| Prednisolone | 50.6 ± 2.24** | 15.7 | 4.8 ± 0.22 | 14.3 |

Administrations were 5 mg/kg i.v. 10 min prior to challenge. Each value indicates the mean ± S.E. of 6 experiments. * and **: Statistical significance from control P<0.05 and P<0.01, respectively.
phylline, in the following decreasing order: isoproterenol > chlorpheniramine > BPS > prednisolone. On the other hand, the amount of the dye leaked was inhibited by the substances, except for BSS, in the following order: chlorpheniramine > isoproterenol > BPS > aminophylline > prednisolone.

**RCA**

The effect of the substances was tested on RCA in rats following an i.d. injection of Rab anti-rat. Rats were administered 5 mg/kg of the substances i.v. at 10 min prior to the antiserum. As shown in Table 3, the manifestation of edema was decreased with all substances. Isoproterenol showed the most potent activity with a 38.7% inhibition, followed by BPS with 29.3%. The others were in a range from 19.6 to 27.4%.

| Substance    | % Inhibition |
|--------------|--------------|
| BPS          | 29.3±2.37    |
| BSS          | 22.2±7.61    |
| Aminophylline| 22.1±3.64    |
| Chlorpheniramine| 19.6±2.16  |
| Isoproterenol| 38.7±3.08    |
| Prednisolone | 27.4±3.15    |

Administrations were 5 mg/kg i.v. at 10 min prior to challenge. Each value indicates the mean±S.E. of 6 experiments, except for 9 experiments with BPS.

Subsequently, in order to determine the time course of the activity of BPS and BSS, these substances were administered in an equal molar dose corresponding to 200 mg/kg of baicaline p.o. and i.p. at varying times prior to the anti-serum. As shown in Figs. 2 and 3, with oral administration, the maximum at 8 hr was 26.3±0.99% inhibition with baicaline, at 6 hr was 31.8±5.48% inhibition with BPS and 23.1±2.93% inhibition with BSS respectively. On the other hand, in the case of the i.p. route, the maximum at 8 hr was 32.4±
Inhibitory activity of substances tested in a concentration of $10^{-4}$ g/ml on the Schultz-Dale reaction is shown in Table 4. A fairly large difference was observed in their activities in the ileum and tracheal muscle. In the ileum, chlorpheniramine and isoproterenol inhibited the reaction completely. BSS and aminophylline, however, showed little or no effect. BPS gave a 53.5% inhibition which corresponded to about one half that of chlorpheniramine. On the tracheal muscle, the effect of BPS was 75.6% inhibition, while aminophylline and/or isoproterenol alone relaxed the muscle below the base line and showed more

| Substance         | % Inhibition |
|-------------------|--------------|
|                   | Ileum       | Tracheal muscle |
| BPS               | 53.5±5.60   | 75.6±6.16       |
| BSS               | 32.3±9.23   | 52.8±11.39      |
| Aminophylline     | 11.2±4.97   | 133.6±16.89     |
| Chlorpheniramine  | 107.4±4.01  | 56.5±5.32       |
| Isoproterenol     | 94.9±1.54   | 128.1±6.20      |
| Prednisolone      |             | 45.1±20.19      |

Percent inhibition was calculated by the following formula:

$$1 - \frac{A'}{A/H} \times 100.$$  

$H$ and $A$ are contraction magnitudes by histamine (ileum: $10^{-7}$ g/ml), tracheal muscle: $10^{-6}$g/ml) and $10^{-4}$g/ml antigen respectively. $H'$ is the contraction magnitude by histamine prior to addition of the substance and $A'$ is that by the antigen in the presence of the substance. Each value indicates the mean ± S.E. of 4 experiments.
than 100% inhibition. The others inhibited by about 50%.

Arthus reaction

BPS and BSS were administered in a dose of 100 mg/kg i.p. 2 hr prior to challenge into rabbits, and 5 mg/kg of chlorpheniramine was also used as a comparative. The result obtained with substances tested on the Arthus reaction is shown in Fig. 4. The inflammatory reaction, mainly edema and redness, appeared within 1 hr and reached a maximum at 20 to 24 hr after the challenge. With the exception of a slight inhibition by BPS at 2 to 4 hr after the antigen, the other substances hardly affected the Arthus reaction.

Forssman cutaneous vasculitis

The effect of substances tested on the Forssman cutaneous vasculitis is shown in Fig. 5. The substances were administered i.v. at 10 min prior to challenge, with 5 to 10 mg/kg of BPS and BSS, the dimension of the dye leakage as a result of Forssman reaction was inhibited by 25 to 30%, and also inhibited with 5 mg/kg of chlorpheniramine by about 40%. On the other hand, the amount of the dye leakage was inhibited with these substances by 18 to 34% except for 5 mg/kg dosage of BPS.
**Experimental asthma**

To guinea pigs sensitized passively with Rab anti-Ea, an i.v. injection of the antigen caused a transient increase followed by a persistent decrease in the rate of respiration, and a persistent decrease in the depth of respiration continued for more than 30 min. A considerable fall in blood pressure was also observed, the maximum being about 60%. BPS and BSS were given in 3 different doses i.v. at 15 min before challenge. Five mg/kg of BPS prevented a decrease in the depth and there was a slight decrease in the rate of respiration. A fall in blood pressure was also prevented by BPS. The same dose of BSS, however, failed to prevent the respiratory disorder or a fall in blood pressure. In doses of 10 to 20 mg/kg, BPS and BSS inhibited the hypotension and also the anaphylactic changes in respiration, especially the decrease in depth (Fig. 6).

**DISCUSSION**

In previous papers, the authors reported the inhibitory effects of baicalin and baicalein on active and passive anaphylaxis as well as results on experimental asthma. In the present paper, the effects of soluble baicalein derivatives, BPS and BSS, on immediate hypersensitivity reactions were studied, and the results obtained were compared with those of certain drugs used as the therapy for asthma. BPS and BSS inhibited heterologous and homologous
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PCAs, RCA, the Forssman cutaneous vasculitis and the Schultz-Dale reaction, but affected little the Arthus reaction.

According to Coombs and Gell (9), immediate hypersensitivity reaction has been classified into three. Type I is atopy and anaphylaxis caused by interaction of reagin (IgE) or anaphylactic antibody and its specific antigen. Type II is cytolysis initiated by the association of humoral antibody and cellular antigen followed by complement fixation. Type III is the Arthus reaction triggered by antigen-antibody complex. PCA and the Schultz-Dale reaction are classified as Type I, while RCA and the Forssman cutaneous vasculitis are type II. From the present data, BPS and BSS inhibited type I and II reactions, but the Arthus reaction belonging to type III was not modified by either BPS or BSS. The effect of BPS was generally more potent than that of BSS, and BPS appears to liberate more easily baicalein than BSS in tissues. Cox (4) introduced DSCG as an effective remedy for asthma. It is a dichromone derivative and selectively blocks the reagin-mediated reaction. In contrast to DSCG, BPS and BSS are conceivable as mono-chromone derivatives, and inhibit either reagin or non-reagin-mediated reaction of type I. In addition, they also inhibited type II reaction. Such being the case, BPS should be extensively applicable for treatment of allergy related diseases. These results give rise to the hope that more excellent drugs can be found among chromone derivatives. On experimental asthma in guinea pigs, BPS and BSS prevented anaphylactic respiratory disorder and a fall in blood pressure. In the present study, passive systemic anaphylaxis (PSA) was employed in guinea pigs, the reason being that PSA is superior in reproducing the reaction.

Recently, many experiments have demonstrated that cyclic AMP plays an important role in controlling anaphylactic mediator release (10-12). Agents which raise cyclic AMP level inhibit histamine and SRS-A release as a result of the antigen-antibody reaction. Isoproterenol increases the intracellular level of cyclic AMP by activating adenylcyclase and theophylline does so by inhibiting the catabolism. In the present study, aminophylline and isoproterenol showed inhibitory actions on both PCAs, RCA and the Schultz-Dale reaction, though there were differences in potency. Inhibition by these drugs can be attributed to an elevation of the level of cyclic AMP.

Movat et al. (13) have reported that antihistaminics inhibited homologous PCA, but not heterologous PCA in guinea pigs. In the present experiment, chlorpheniramine maleate, an antihistaminic, suppressed vasculitis induced by homologous and heterologous PCAs as well as RCA and the Forssman reaction. This discrepancy may be due to species differences or peculiarities of the homocytotropic antibody. As both PCAs and RCA were inhibited by prednisolone, the action is presumably based upon non-specific anti-inflammatory activity. In other experiments, we confirmed that BPS did not potentiate the effect of isoproterenol or aminophylline on anaphylactic mediator release from guinea pig lung (in preparation). In addition, as shown in the present data, BPS had less activity against histamine. Studies are underway regarding the mechanism of the anti-allergic activity of BPS. As well as in the original compound, baicalein, BPS may interfere with the activation of SH-dependent proteolytic enzymes following antigen-antibody reaction and
inhibition of the mediator release would necessarily take place. The soluble derivatives of baicalein, in particular BPS, thus appear worthy of extensive prescription for allergies although absorption with oral dosing would be only moderate.

Acknowledgement: Thanks are due to Takeda Chemical Industries Ltd. for the generous gifts of BPS, BSS and baicalein.

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