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# Scutellariae Radix. X. Inhibitory Effects of Various Flavonoids on Histamine Release from Rat Peritoneal Mast Cells *in Vitro*

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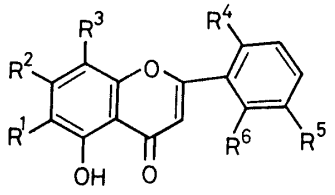
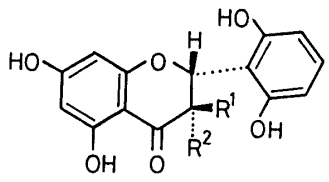
The effects of various flavonoids isolated from the roots of *Scutellaria baicalensis* on the histamine release from mast cells were investigated. Many of the flavonoids (wogonin, wogonin-7-*O*-D-glucuronide, baicalein, skullcapflavone II, (2*S*)-2′,5,6′,7-tetrahydroxyflavanone, (2*R*,3*R*)-2′,3,5,6′,7-pentahydroxyflavanone and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone) inhibited the histamine release from rat mast cells induced by compound 48/80.

**Keywords**——*Scutellaria baicalensis*; Labiatae; histamine; mast cell; flavonoid

Scutellaria Radix ("Ogon" in Japanese), the roots of *Scutellaria baicalensis* GEORGI (Labiatae), has been used in Chinese medicine as a remedy for inflammation, suppurative dermatitis, allergic diseases, hyperlipemia and arteriosclerosis. In the previous papers,[1-4] we reported that baicalein and its derivatives showed antibacterial actions, and improved hyperlipemia and liver injury with the elevation of serum triglyceride and glutamic pyruvic transaminase (GPT) in rats fed peroxidized oil. It was also reported that baicalein, baicalin, two new flavanones [(2*S*)-2′,5,6′,7-tetrahydroxyflavanone and (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone] and a new flavone (2′,5,5′,7-tetrahydroxy-6′,8-dimetoxyflavone)




wogonin: $R^1=R^4=R^5=R^6=H$, $R^2=OH$, $R^3=OCH_3$
wogonin-7-*O*-D-glucuronide: $R^1=R^4=R^5=R^6=H$, $R^2=$*O*-D-glucuronic acid, $R^3=OCH_3$
baicalein: $R^1=R^2=OH$, $R^3=R^4=R^5=R^6=OH$
baicalin: $R^1=OH$, $R^2=$*O*-D-glucuronic acid, $R^3=R^4=R^5=R^6=H$
chrysin: $R^1=R^3=R^4=R^5=R^6=H$, $R^2=OH$
skullcapflavone II: $R^1=R^2=R^3=R^4=OCH_3$, $R^5=H$, $R^6=OH$
2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone: $R^1=H$, $R^2=R^4=R^5=OH$, $R^3=R^6=OCH_3$




(2*S*)-2′,5,6′,7-tetrahydroxyflavanone: $R^1=R^2=H$
(2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone: $R^1=OH$, $R^2=H$

Fig. 1

inhibited the lipid peroxidation induced by $Fe^{2+}$–ascorbic acid, adenosine diphosphate (ADP)–ascorbic acid and reduced nicotineamide adenine dinucleotide phosphate (NADPH)–ADP in rat liver.[3,5,6] Further, among the flavonoids isolated from the roots of *Scutellaria baicalensis*, baicalein and baicalin were reported by Koda *et al.*[7,8] to possess and antiallergic effect.

Thon *et al.*[9] reported that compound 48/80 induced histamine release from rat mast cells. The release of histamine from isolated mast cells is a useful *in vitro* model for studying allergic diseases.[10] Most of the drugs used in the treatment of allergy and asthma (for example, isoprenalline and theophylline) are effective inhibitors of *in vitro* histamine release.[11]

In the present study, we attempted to examine the effects of various flavonoids (Fig. 1) on the histamine release from rat peritoneal mast cells induced by compound 48/80.

## Materials and Methods

**Materials**——The various flavonoids (wogonin, wogonin-7-*O*-D-glucuronide, baicalein, baicalin, chrysin, skullcapflavone II, (2*S*)-2′,5,6′,7-tetrahydroxyflavanone (comp. 1), (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone (comp. 2) and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone (comp. 3) of Scutellariae Radix were isolated by the method described in our previous papers (Fig. 1).[1,5,6]

**Animals**——Male Hertley strain guinea pigs weighing 250—300 g and male Wistar King strain rats weighing 150—200 g were housed in a room maintained at $25 \pm 1$ °C with $60 \pm 10\%$ relative humidity and given free access to food and water for at least 1 week before experiments. The room was illuminated for 12 h a day starting at 7:00 a.m.

**Measurement of Histamine-Induced Contraction in the Isolated Guinea Pig Ileum**——Guinea pigs were sacrificed by means of a blow on the head and the ileum was isolated. A length of 1.5 to 2.0 cm was suspended in Locke–Ringer's solution bubbled through with $O_2$ gas (containing 5% $CO_2$) in an organ bath kept at 37 °C. Ileum contractions were isotonically recorded by means of a lever loaded with 0.5 g on a smoke drum. The ileum was preincubated with a test flavonoid or diphenhydramine HCl for 1 min, and then histamine ($10^{-7}$ g/ml, final concentration) was added and incubation was carried out for 3 min at 37 °C. The extent of contraction was estimated from the maximal contraction of the isolated ileum after addition of histamine. The activity of various flavonoids or diphenhydramine HCl on histamine-induced contractions in the isolated ileum was expressed as the 50% inhibition concentration ($IC_{50}$ μM, Litchfield–Wilcoxon method).

**Preparation of Mast Cells**——Mast cells were isolated from the peritoneal cavity fluid of normal Wistar rats weighing 150—200 g by a slight modification of the method described by Chakravarty and Zeuthen.[12] The cells were suspended in ice-cold phosphate-buffered saline (PBS, pH 7.0) of Dulbecco and Vogt[13] containing heparin (10 U/ml), then adjusted to 20% with Ficoll, and layered on 30 and 40% Ficoll in a siliconized glass centrifuge tube. After centrifugation at $400 \times g$ and 4 °C for 15 min, the layer containing mast cells was pipetted out. The cells were washed three times with 5 ml of PBS (pH 7.0) and suspended in the same medium at $3.0 \times 10^6$ cells/ml. The cell suspensions contained 90% or more viable mast cells, as determined by the toluidine blue (0.1% in 50% ethanol) staining test of Bray and Van Arsdel.[14]

**Measurement of Histamine Release from Mast Cells Induced by Compound 48/80**——The flavonoids or disodium cromoglycate (DSCG) were added to mast cell suspension (1.8 ml). After 1 min, 0.1 ml of compound 48/80 solution (10 μg/ml, 0.1 ml) was added, and the mixture was incubated at 37 °C for 10 min in a final volume of 2 ml. The reaction was terminated by cooling the mixture on ice. The mixture was centrifuged at $630 \times g$ for 5 min, then histamine in the supernatant fluid was assayed fluorometrically according to the method of Shore *et al.*[15] The total content of histamine in intact cells was regarded as the amount of the amine extracted from the cells which had been frozen and thawed five times.[16] The amount of histamine released from mast cells by compound 48/80 alone was taken as 100%, and the percent inhibition by each flavonoid was calculated on that basis. The activity of various flavonoids or DSCG on histamine release from mast cells induced by compound 48/80 was expressed as the 50% inhibition concentration ($IC_{50}$ μM, Litchfield–Wilcoxon method).

## Results

### Effects of Various Flavonoids on Histamine-Induced Contraction in the Isolated Guinea Pig Ileum

The $IC_{50}$ value of diphenhydramine HCl for histamine-induced contraction in the isolated ileum was 8 μM. Wogonin, wogonin-7-*O*-D-glucuronide, baicalein, baicalin, chrysin, skullcapflavone II, comp. 1, comp. 2 and comp. 3 (at 10, 50, 100 and 200 μM) had no effect on

TABLE I. Effects of Various Flavonoids and DSCG on Histamine Release from Mast Cells Induced by Compound 48/80

| Compound | Concentration ($\mu$M) | Inhibition (%) | $IC_{50}$ (95% C.L., $\mu$M) |
|---|---|---|---|
| Wogonin | 10 | 10.1 | |
| | 25 | 17.8 | 40.0 (20.4—78.5) |
| | 50 | 62.2 | |
| | 100 | 82.3 | |
| Wogonin-7-*O*-D-glucuronide | 10 | 3.3 | |
| | 25 | 5.2 | |
| | 50 | 10.1 | 140.0 (57.1—343.0) |
| | 100 | 46.4 | |
| | 200 | 67.7 | |
| Baicalein | 10 | 6.9 | |
| | 25 | 19.0 | 52.1 (26.5—102.0) |
| | 50 | 51.2 | |
| | 100 | 74.0 | |
| Baicalin | 50 | 3.2 | |
| | 100 | 12.3 | >200 |
| | 200 | 15.4 | |
| Chrysin | 50 | 1.8 | |
| | 100 | 15.3 | >200 |
| | 200 | 20.7 | |
| Scullcapflavone II | 10 | 4.8 | |
| | 25 | 65.7 | 15.0 (9.9—22.7) |
| | 50 | 95.3 | |
| | 100 | 98.2 | |
| (2*S*)-2′,5,6′,7-Tetrahydroxyflavanone | 5 | 5.3 | |
| | 10 | 40.8 | 17.7 (7.0—44.5) |
| | 25 | 72.4 | |
| | 50 | 80.3 | |
| (2*R*, 3*R*)-2′,3,5,6′,7-Pentahydroxyflavanone | 5 | 7.2 | 15.5 (9.1—26.4) |
| | 10 | 17.8 | |
| | 25 | 60.3 | |
| | 50 | 98.0 | |
| 2′,5,5′,7-Tetrahydroxy-6′,8-dimethoxyflavone | 5 | 2.3 | |
| | 10 | 23.0 | 19.5 (11.2—34.1) |
| | 25 | 66.9 | |
| | 50 | 78.9 | |
| DSCG | 10 | 5.8 | |
| | 25 | 85.0 | 17.5 (12.3—24.9) |
| | 50 | 98.4 | |
| | 100 | 99.3 | |

$N=5$.

histamine-induced contraction in the isolated ileum of guinea pigs.

**Inhibitory Effects of Various Flavonoids on Histamine Release from Mast Cells Induced by Compound 48/80**

The histamine release of the control without addition of any test solution was 54.8%. As shown in Table I, the $IC_{50}$ values of wogonin, wogonin-7-*O*-D-glucuronide, baicalein, skullcapflavone II, comp. 1, comp. 2, comp. 3 and DSCG were 40.0, 140.0, 52.1, 15.0, 17.7, 15.5, 19.5 and 17.5 $\mu$M, respectively. Baicalin and chrysin had no effect on the histamine release from mast cells induced by compound 48/80.

## Discussion

The effects of various flavonoids isolated from the roots of *Scutellaria baicalensis* ("Ogon" in Japanese) on the histamine-induced contraction in the isolated ileum of guinea pigs and on the histamine release from mast cells induced by compound 48/80 were investigated. It is well known that a primary step in allergic reaction and inflammatory reaction is the release of histamine. In the present experiments, it was found that the flavonoids tested [wogonin, wogonin-7-*O*-D-glucuronide, baicalein, baicalin, chrysin, skullcapflavone II, (2*S*)-2′,5,6′,7-tetrahydroxyflavanone, (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone] had no effect on the histamine-induced contraction in the isolated ileum of guinea pigs.

It is well known that compound 48/80 causes histamine release from mast cells by injuring the cell membrane.[9] The action of compound 48/80 does not involve an immunological mechanism, does not require the presence of $Ca^{2+}$ in the extracellular medium and is not enhanced by the addition of phosphatidylserine.[17]

The flavonoids tested, except baicalin and chrysin, inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80. It is noteworthy that skullcapflavone II, (2*S*)-2′,5,6′,7-tetrahydroxyflavanone, (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone show almost the same $IC_{50}$ values as DSCG in the inhibition of histamine release from mast cells induced by compound 48/80. It was found that the inhibitory activities of the three new compounds, (2*S*)-2′,5,6′,7-tetrahydroxyflavanone, (2*R*, 3*R*)-2′,3,5,6′,7-pentahydroxyflavanone and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone, and a known compound, skullcapflavone II, on the histamine release from mast cells induced by compound 48/80 were stronger than that of baicalein, which was reported to show antiallergic actions by Koda *et al.*[7,8] These results suggest that hydroxyl groups or methoxyl groups in the B ring of the flavanone and flavone skeleton may be essential for inhibitory activity on the histamine release from mast cells. The above four compounds may be useful clinically as antiallergic drugs.

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