Anti-5-hydroxytryptamine₃ Effect of Galanolactone, Diterpenoid Isolated from Ginger

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It has been reported that an acetone extract of ginger and its fractions have anti-5-HT (5-hydroxytryptamine; serotonin) effects. In the present study, guinea pig ileum, rat stomach fundus and rabbit aortic strips are used in order to determine the constituents of fraction 2 which are responsible for anti-5-HT effect and to examine their pharmacological properties.

The analysis of fraction 2—3 indicated that galanolactone, a diterpenoid, is one of the active constituents. In guinea pig ileum, galanolactone inhibited contractile responses to 5-HT with a pIC₅₀ value 4.93. pIC₅₀ value of galanolactone against the response to 2-methyl-5-HT, a selective 5-HT₃ agonist, in the presence of methysergide at 1 × 10⁻³ M was 5.10. pIC₅₀ values of ICS 205-930, a selective 5-HT₃ antagonist, were 5.50 and 7.49, respectively. The concentration-response curve of 5-HT was shown as a biphasic curve and galanolactone caused a selective shift to the right of the second phase.

In the same preparations, the pIC₅₀ value of galanolactone and ICS 205-930 against the response to carbachol (CCh) was 4.45 and 4.46. The inhibitory effect of galanolactone on the 5-HT response in the stomach fundus and aortic strips was less than that in the ileum.

In addition, in the thoracic aorta precontracted with 50 mM K⁺, the relaxing effect of galanolactone was about 1/10 of that of papaverine.

These results suggest that the anti-5-HT effect of galanolactone, a diterpenoid isolated from ginger, is related to antagonism of 5-HT₃ receptors.

Keywords ginger; diterpenoid; galanolactone; anti-serotonergic action; 5-hydroxytryptamine, receptor

Introduction

We have already reported the inhibitory effect of acetone extract, and several constituents of ginger on the responses to serotonin (5-hydroxytryptamine, 5-HT). ¹ In case of vomiting, ginger may be used singly or in combination with the tubers of Pinellia ternata in China and Japan. The anti-emetic effects are generally held to reflect its dopamine D₂ receptor blocking activity, recent data has indicated that there is a good correlation between 5-HT₃ receptor antagonist potency and anti-emetic efficacy. ² Further experiments were conducted to clarify the anti-5-HT constituents and the existence of anti-5-HT₃ antagonistic potency.

Experimental Methods

Fractionation Ginger was purchased from local market in Osaka and the roots, were coarsely cut, soaked in three times the volume of acetone for 2d. The filtrate was concentrated under reduced pressure below 40°C, dried and kept in a desicator. Fractionation of the active constituents was carried out by our method, as shown Fig. 1. Fractions 2 and 3 significantly inhibited the contractile response induced by 5-HT. The active constituents of fraction 3 have been reported ³ The present experiments were conducted in order to clarify the active constituents of fraction 2. Fraction 2 was further separated by silica gel column chromatography (Merck, Silica gel 60 F-254, elution fluid: gradient, hexane: ethylacetate = 10:1 to 3:1), to obtain fractions 2-1 to 2-4. Fraction 2-3 was similarly purified by silica gel silanised column chromatography (Merck, silica gel silanised, elution fluid, 50% methanol), comparison of mass spectrum (MS), proton nuclear magnetic resonance ('H-NMR) and infrared spectrum (IR) with those of the standards indicated that the chief ingredient of fraction 2-3 was galanolactone ⁴ (Fig. 2).

Recording of Response Male Hartley guinea pigs (about 300 g) were bled to death by severing both arteries and the ileum removed and cut into strips (10—15 mm). Rat stomach fundus and rabbit thoracic aorta were also isolated with the same technique as the ileum. The fundus was cut into strips (1×5 mm) and the aorta was made the spiral cord and cut into strips (2×10 mm). Each ileum strip was placed in a tissue bath containing 25 ml of Tyrode solution, maintained at 37°C and stomach fundus strips and aortic strips in the Krebs Henseleit solution.

The compositions of Tyrode solution and Krebs Henseleit solution were as follows (mm): Tyrode; NaCl 137.9, KCl 2.7, CaCl₂ 1.8, MgCl₂, NaH₂PO₄ 1.1, NaHCO₃ 11.9, glucose 5.6. Krebs Henseleit: NaCl 118.1, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25.0, MgSO₄ 1.2, glucose 10.0. It was aerated with a 95%O₂-5%CO₂ gas mixture and kept at pH 7.4. An initial stretch

ginger (4.96 kg)
extracted with acetone evaporated
acetone extract (167.5 g, 3.4%)
SiO₂ column chromatography solvent: n-hexane : ethyl acetate (30:1—10:1)
Fr. 1 (14%) Fr. 2 (12%) Fr. 3 (50%) Fr. 4 (15%)
thin layer chromatogram
acetone ext.
Fr. 1 Fr. 2 Fr. 3 Fr. 4
0 0 0
0 0 0 0
0 0 0 0
0 0 0 0

solvent : n-hexane : ethyl acetate (10 : 1)
plate : pre-coated Silica gel 60 F₂₅₄
spray : 1% Ce(SO₄)₂/10% H₂SO₄, heat

Fig. 1. Flow Diagram of Fractionation of Ginger Acetone Ext.
tension of 1 g was applied to each strip and at least 1 h was allowed before the start of the experiment. The contractile response of 5-HT was obtained by addition of 5-HT to the bath at concentrations of 10⁻⁶ M. Each tissue was then washed three times every 10 min. Thirty minutes thereafter, the contractile response of 5-HT (10⁻⁵ M) was again obtained 20 min after the application of test drugs. The contraction to 5-HT was compared to the response obtained in the first contraction of 5-HT taken as 100%. Non-cumulative concentration-response curve for 5-HT were established by adding increasing concentrations of the agonist to the organ bath at intervals of at least 15 min to avoid tachyphylaxis. Each concentration was left in contact with the tissue for 1 min. Antagonists were preequilibrated for 10 min prior to addition of 5-HT. The contraction expressed as percentage of the maximal response to 5-HT obtained from several preparations were plotted as mean value in order to obtain a log-concentration-response curve.

Contractions were measured for a force transducer (Nihon Denki San-ei: 45196A) and recorded on an oscillograph (Nihon Denki San-ei: 363).

**Drugs Treatment** Test samples were dissolved in ethanol or dimethyl sulfoxide (DMSO) and diluted in distilled water. The final bath concentration of ethanol and DMSO were less than 0.01%. Control experiments indicated that the concentration of ethanol and DMSO did not have any effect on the response of the ileum strips.

Drugs used were serotonin-creatine sulfate (5-HT, Wako Pure Chemical Industries Ltd., Osaka), carbamylcholine (CCH, Aldrich Chemical Company, Inc. Milwaukee) methyserygide (Sandoz, Switzerland) ketanserin and ICS 205-930 (Research Biochemical Inc. Massachusetts). 2-Methylserotonin was synthesized in our laboratory. Statistical analysis was performed by Dunnett's method.

**Results**

The Effect of Fractions on the Contraction of 5-HT in Guinea Pig Ileum It was shown that fraction 2, and fractions 2-1, 2-2, 2-3, 2-4 exhibited an anti-5-HT effect and fractions 2-1, 2-2 and 2-3 had nearly equal potency of inhibitory effects (Fig. 3).

The Effect of Galanolactone on the Concentration-Response Curve of 5-HT in Guinea Pig Ileum As shown in Fig. 4, the concentration-response curve of 5-HT was given as a biphasic curve. The effects of galanolactone (3×10⁻⁶ and 1×10⁻⁵ M) were found to possess an anti-5-HT effect and the first phase of the response curve was not affected by galanolactone (Fig. 4).

The Effect of Galanolactone on the Contraction to Cumulative Treatment of CCH in Guinea Pig Ileum As shown in Fig. 5, galanolactone (1×10⁻⁵ and 3×10⁻⁵ M) possessed a potency of anti-cholinergic effect, while the potency was weaker than that of anti-5-HT effect (Fig. 5).

The Relaxing Effect of Galanolactone on the High-concentration K⁺ Contraction in Rabbit Thoracic Aorta As shown in Fig. 6, the relaxing effect of galanolactone was approximately 1/10 that of papaverine (Fig. 6).

Values of pIC₅₀ of Galanolactone, Methysergide, Ketanserin and ICS 205-930 on the Contraction by 5-HT in Rat Stomach Fundus and Rabbit Thoracic Aorta and the Contraction by 2-Methyl-5-HT with Pretreatment of Methysergide in Guinea Pig Ileum In rat stomach fundus, 5-Hydroxytryptamine (−log M)

Fig. 4. Effect of Galanolactone on the Concentration-Response Curve of 5-HT in the Guinea Pig Ileum ○, control; ●, galanolactone 3 × 10⁻⁴ M; ▲, 1 × 10⁻⁴ M. Each point indicates the mean of six experiments. Vertical bars indicate the S.E. of the mean.

Carbachol (−log M)

Fig. 5. Effect of Galanolactone on the Concentration-Response Curve of CCH in the Guinea Pig Ileum ○, control; ●, galanolactone 1 × 10⁻⁵ M; ▲, 3 × 10⁻⁵ M. Each point indicates the mean of six experiments. Vertical bars indicate the S.E. of the mean.
Fig. 6. The Relaxing Effect of Galanolactone (●) and Papaverine (○) on the High-concentration K⁺ Contraction in the Rabbit Thoracic Aorta. Each point indicates the mean of six experiments. Vertical bars indicate the S.E. of the mean.

### Table I. Inhibitory Effect of Galanolactone, Methysergide, Ketanserin and ICS 205-930 on the Constrictor Responses of Isolated Guinea Pig Ileum Induced by 2-Methyl-5-HT and Rabbit Thoracic Aorta and Rat Stomach Fundus Induced by 5-HT

<table>
<thead>
<tr>
<th>Compounds</th>
<th>n</th>
<th>pIC₅₀</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rat S.F.</td>
<td>Rabbit T.A.</td>
<td>G.P Ileum</td>
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<tr>
<td>Galanolactone</td>
<td>6</td>
<td>4.00</td>
<td>4.00</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>ICS 205-930</td>
<td>6</td>
<td>4.00</td>
<td>4.00</td>
<td>7.49</td>
<td></td>
</tr>
<tr>
<td>Methysergide</td>
<td>6</td>
<td>7.64</td>
<td>6.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketanserin</td>
<td>6</td>
<td>4.00</td>
<td>6.71</td>
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</table>

The contractile response of guinea pig ileum was induced by 2-methyl-5-HT (1 x 10⁻⁴ M) and each drug was treated 5 min after the treatment of methysergide (1 x 10⁻⁴ M). The contractile response of rabbit T.A. and rat S.F. was induced by 5-HT (1 x 10⁻⁴ M). pIC₅₀ is the negative logarithm of the concentration (M) of galanolactone, ICS 205-930, methysergide and ketanserin required to prevent 50% of contractile response elicited by 2-methyl-5-HT and 5-HT.

### Table II. Inhibitory Effect of Galanolactone, ICS 205-930, Atropine and Ketanserin on the 2-Methyl-5-HT, 5-HT and CCh Induced Contraction of Isolated Guinea Pig Ileum

<table>
<thead>
<tr>
<th>Compounds</th>
<th>n</th>
<th>pIC₅₀</th>
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<tr>
<td></td>
<td></td>
<td>2-Methyl-5-HT</td>
<td>5-HT</td>
<td>CCh</td>
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<td>Galanolactone</td>
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<td>ICS 205-930</td>
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<td>5.30</td>
<td>4.46</td>
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<tr>
<td>Atropine</td>
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<td>5.46</td>
<td>8.35</td>
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<tr>
<td>Ketanserin</td>
<td>6</td>
<td>4.74</td>
<td>5.15</td>
<td>4.13</td>
<td></td>
</tr>
</tbody>
</table>

On the contractile response induced by 2-methyl-5-HT, each drug was treated 5 min after the pretreatment of methysergide (1 x 10⁻⁴ M). pIC₅₀ is the negative logarithm of the concentrations (M) of galanolactone, ICS 205-930, atropine and ketanserin required to prevent 50% of contractile response elicited by 2-methyl-5-HT (1 x 10⁻⁴ M), 5-HT (1 x 10⁻⁴ M) and CCh (3 x 10⁻⁴ M).

the value of pIC₅₀ of galanolactone was more than 4.00 and was also ketanserin, while that of methysergide was 7.64. In rabbit thoracic aorta, the value of galanolactone was more than 4.00 and that of ketanserin was 6.71. In guinea pig ileum, the value of galanolactone was 5.10 and that of ICS 205-930 was 7.49 (Table I).

**Comparison of the Value of pIC₅₀ of the Galanolactone between the Contraction by 2-Methyl-5-HT with Pretreatment of Methysergide and the Contraction by CCh in Guinea Pig Ileum**

The value of pIC₅₀ of the galanolactone on the contraction by 2-methyl-5-HT was 5.10, while the value on the contraction by CCh was 4.45. Each value of ICS 205-930 and atropine were 7.94, 4.46 and 6.34, 8.35, respectively (Table II).

### Discussion

Ginger is one of the important natural stomachics. Anti-emetic effect of ginger has also been known from ancient times. In recent years, the relationship between the 5-HT₃ receptor antagonist and anti-emetic and gastrointestinal motility enhancing effect has been largely recognized. In the present study, one of the main aims was to determine the presence of 5-HT₃ antagonism in ginger constituents in order to substantiate the known medicinal efficacy. This is the first report which identified the anti-5-HT effect of galanolactone, a diterpenoid. Results indicated that anti-5-HT effect of galanolactone was much greater in a guinea pig ileum, which has mainly 5-HT₃ receptor, than that in rat fundus strips, which are known for the much presence of 5-HT₁ receptors, and rabbit aorta strips, which contain mainly 5-HT₂ receptors. In addition, the effect of galanolactone was much greater in response to a selective 5-HT₃ agonist, 2-methyl-5-HT, in the presence of a selective 5-HT₁ and 5-HT₂ antagonist, methysergide. Recently it was reported that at a low concentration (below 3 x 10⁻⁴ M), contractile response of 5-HT can be mediated by release of substance P which subsequently releases acetylcholine. The value of pIC₅₀ of galanolactone on the contraction of CCh was 4.45, while the value on the contraction of 2-methyl-5-HT with pretreatment of methysergide was 5.10. ICS 205-930 caused a selective shift to the right of the second phase of the 5-HT curve. Galanolactone also shifted to the right of the second phase of the concentration-response curve selectively. The results suggest that 5-HT₃ antagonism is involved in the anti-5-HT action of galanolactone.

The results in the present study not only substantiate the known medicinal efficacy of ginger but also may help to develop a new type of anti-5-HT₃. Further experiments are currently in progress to examine the in vivo effect of galanolactone and to determine the active constituent in other fractions of ginger.

### References