EFFECTS ON GASTRIC ACID SECRETION OF A STEROIDAL ALKALOID, EPIPACHYSAMINE-A, EXTRACTED FROM PACHYSANDRA TERMINALIS SIEB. ET ZUCC.

MASAKI MAEDA-HAGIWARA,* KAZUO WATANABE,** HIROSHI WATANABE, MASAO SHIMIZU AND TOHRU KIKUCHI*

Research Institute for Wakan-yaku, Toyama Medical & Pharmaceutical University,* Toyama, 930-01, Japan and Department of Drug Evaluation and Toxicological Sciences, Faculty of Pharmaceutical Sciences, Chiba University,** 1-33 Yayoi-cho, Chiba, 260, Japan

(Received October 26, 1983)

Effect of a steroidal alkaloid, epipachysamine-A, extracted from Pachysandra terminalis Sieb. et Zucc. on gastric acid secretion was studied in rats. Epipachysamine-A prevented 2-deoxy-D-glucose- or thyrotropin-releasing hormone-stimulated gastric acid secretion in anesthetized rats. However, the compound did not influence betahanechol- or electrical vagal stimulation-induced gastric acid secretion. These results suggest that the effect of epipachysamine-A is due to the influence on the central nervous regulatory mechanism in the gastric acid secretion.

Keywords — gastric acid secretion; epipachysamine-A; Pachysandra terminalis; 2-deoxy-D-glucose; thyrotropin-releasing hormone

INTRODUCTION
Pharmacological studies of central nervous system (CNS) control of gastric function have been performed and the understanding of the peptidergic and monoaminergic regulation of gastric acid secretion has been advanced (see review by Morley et al.). We have studied the effects of Japanese traditional medicine on gastrointestinal functions and found that magnolol (neolignane derivative, one of the active compound of Magnolia officinalis REHDER et WILSON) prevented the stress ulcers and the gastric acid secretion via CNS.

Fruit of Pachysandra terminalis Sieb. et Zucc. (Japanese name Fukki-sô) (Buxaceae) has been used for treatment of gastrointestinal disorders in Ainu’s folk medicine, a part of Japanese traditional medicine. Many kinds of new steroidal alkaloids of pregnane type were isolated in crystalline form from the plant. The pachysandra alkaloids produced behavioral changes due to CNS excitation.

The present study was undertaken to evaluate the effect of epipachysamine-A, one of the pachysandra alkaloids, on gastric acid secretion centrally stimulated by 2-deoxy-D-glucose or thyrotropin-releasing hormone (TRH) and peripherally stimulated by betahanechol or vagus nerve in rats.

METHODS AND MATERIALS
Gastric Acid Secretion — Male Wistar rats (ST. substrain from Saky Lab. Co., Ltd.), weighing 200—250 g, were anesthetized with urethane (1.25 g/kg i.p.) after a 24-h fast, but were allowed free access to water. A gastric acid secretion assay was performed as described previously with a slight modification. The total amount of the secreted acid was expressed in terms of μeq HCl/30 (vagal stimulation), 60 (TRH administration) or 120 (2-deoxy-D-glucose- or betahanechol-stimulation) min per animal. Basal secretion was low and almost constant during the experimental periods. Therefore, the data in-
dicate values in which the corresponding basal secretion before treatment was deducted from the acid output due to the treatment.

Gastric acid secretion was stimulated with 200 mg/kg \textit{i.v.} of 2-deoxy-D glucose (2DG), 10 \( \mu g/\text{rat} \ \textit{i.c.v.} \) of TRH, 0.5 mg/kg \textit{s.c.} of bethanecol and by vagus nerve stimulation. Intracerebroventricular administration (\textit{i.c.v.}) of TRH\textsuperscript{3} and vagus nerve stimulation\textsuperscript{5} were done according to the procedure described previously. Vagal stimulation was repeated three times at about 60 min intervals. The second and third stimulation produced constant increase in acid output, so drugs were administered 15 min before the 3rd stimulation.

\textbf{Drugs} — Drugs except epipachysamine-A (EPI-A, 1\% gum arabic solution) dissolved in a saline solution. EPI-A and atropine were intraperitoneally administered 15 min before the injection of 2DG, bethanecol, 2nd TRH and 3rd vagal stimulation, respectively. All drugs except TRH (10 \( \mu l/\text{rat} \) were administered in a 1 ml/kg volume. When the drug is a salt, the weight refers to that of the salt. Since there was no difference in the response between by gum arabic solution and by saline solution, control was composed of both vehicle values.

Fig. 1 shows the chemical structure of EPI-A, which is purified from the plant according to the method of Tomita \textit{et al.}\textsuperscript{3} Atropine sulfate (Wako), bethanecol (Eisai), 2-deoxy-D-glucose (Nakarai), thyrotropin-releasing hormone (Protein Res. Foundation) and urethane (Wako) were used.

\textbf{Statistical Analysis} — All data are presented as means±S.E. The data were analyzed by Student’s \textit{t-test} or paired \textit{t-test}.

\textbf{RESULTS}

\textit{Gastric Acid Secretion Induced by Central Stimulation}

2DG (200 mg/kg \textit{i.v.}) stimulated basal acid secretion about 30 min after its administration and the effect lasted for more than 2 h. Total acid output for 2 h was about 140 \( \mu e q \) HCl (Table I). Pretreatment with EPI-A (25 and 50 mg/kg \textit{i.p.}) inhibited significantly this response up to 2 h following the 2DG injection. Atropine (0.1 mg/kg \textit{i.p.}) also prevented strongly 2DG-stimulated acid secretion but did not reduce the acid secretory response below the basal line (Table I).

Repeated administration of TRH (10 \( \mu g/\text{rat} \ \textit{i.c.v.} \) induced reproducible acid secretion which lasted for more than 1 h (Fig. 2 and Table II). Pretreatment with EPI-A (25 mg/kg \textit{i.p.}) inhibited the 2nd TRH-induced gastric acid secretion as shown in Fig. 2. Table II shows the inhibitory effects of EPI-A on the gastric response for 1 h by the second TRH injection. Atropine (0.1 mg/kg \textit{i.p.}) abolished the TRH-induced acid secretion.

\textit{Gastric Acid Secretion Induced by Peripheral Stimulation}

Basal acid secretion was low in rats with the left vagus nerve cut (5.3±2.0 \( \mu e q \) HCl/30 min, \( n = 12 \)). Electrical vagus stimulation for 10 min led to only short (about 25—30 min) but reproducible acid secretion. Bethanecol (0.5 mg/kg \textit{s.c.}) stimulated basal gastric acid secretion about 10 min after its administration and the effect lasted for more than 2 h. Table III summarizes the effects of EPI-A on vagal stimulation- or bethanecol-induced acid secretion. Pretreatment with EPI-A (50 mg/kg \textit{i.p.}), which inhibited significantly the 2DG- or TRH-induced acid secretion, had no significant influence on the vagal- or bethanecol-stimulated acid secretion, while atropine (0.1 mg/kg \textit{i.p.}) prevented strongly those stimulated acid secretions.
TABLE I. Effect of EPI-A on 2-Deoxy-d-Glucose-Stimulated Gastric Acid Secretion in Rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose mg/kg</th>
<th>No. of rat</th>
<th>Increase in acid output μeq HCl/2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DG</td>
<td>200 i.v.</td>
<td>12</td>
<td>143.0 ± 24.9</td>
</tr>
<tr>
<td>+ EPI-A</td>
<td>12.5 i.p.</td>
<td>6</td>
<td>153.1 ± 36.6</td>
</tr>
<tr>
<td></td>
<td>25.0 i.p.</td>
<td>6</td>
<td>51.2 ± 17.1a</td>
</tr>
<tr>
<td></td>
<td>50.0 i.p.</td>
<td>6</td>
<td>53.8 ± 25.8a</td>
</tr>
<tr>
<td>+ Atropine</td>
<td>0.1 i.p.</td>
<td>6</td>
<td>23.3 ± 7.6b</td>
</tr>
</tbody>
</table>

The values are means ± S.E.M. (the acid output after drug administration minus basal values). Basal secretion is 11.6 ± 1.4 μeq HCl/30 min, n = 36. Epipachysamine-A (EPI-A) and atropine were given i.p. 15 min before the injection of 2-deoxy-d-glucose (2DG). a) p < 0.05, b) p < 0.01 vs. 2DG.

FIG. 2. Influence of EPI-A on TRH-Induced Gastric Acid Secretion in Rats

Thyrotropin-releasing hormone (TRH, 10 μg/rat) was injected twice into the cerebroventricle. Epipachysamine-A (EPI-A, 25 mg/kg i.p.) was given 15 min before the 2nd TRH injection.

DISCUSSION

EPI-A inhibited 2DG- or TRH-induced acid secretion while the compound did not influence electrical vagus stimulation- or betahanechol-induced acid secretion. These results indicate that effect of EPI-A is due to the influence on the central nervous system. The present study is the first that indicated the centrally acting effect of this type of alkaloid in the gastric functions. Magnolol (neolignane derivative), one of the active compound of Magnolia officinalis REHDER et WILSON, significantly prevents
TABLE II.  Effect of EPI-A on TRH-Stimulated Gastric Acid Secretion in Rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg i.p.)</th>
<th>No. of rats</th>
<th>Increase in acid output (μeq HCl/60 min) Before drug</th>
<th>After drug</th>
<th>Student’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml/kg</td>
<td>8</td>
<td>101.6 ± 18.0</td>
<td>131.1 ± 12.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>EPI-A</td>
<td>25</td>
<td>4</td>
<td>98.7 ± 23.8</td>
<td>67.0 ± 29.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4</td>
<td>102.4 ± 29.9</td>
<td>35.6 ± 9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.1</td>
<td>4</td>
<td>102.8 ± 22.1</td>
<td>4.5 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Thyrotropin-releasing hormone (TRH, 10 μg/rat i.c.v.) was given twice (before and after drug administration) at about 2 h intervals. EPI-A and atropine were administered i.p. 15 min before the 2nd TRH injection. <sup>a</sup>p < 0.05 vs. corresponding before drug control (paired t-test).

TABLE III. Effects of EPI-A on Vagus Nerve Stimulation- or Bethanechol-Induced Gastric Acid Secretion in Rats.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg i.p.)</th>
<th>Vagus stimulation (μeq HCl/30 min)</th>
<th>Bethanechol (μeq HCl/2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml/kg</td>
<td>44.7 ± 7.9 (4)</td>
<td>210.5 ± 38.6 (5)</td>
</tr>
<tr>
<td>EPI-A</td>
<td>50</td>
<td>34.3 ± 5.3 (4)</td>
<td>205.6 ± 53.8 (5)</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.1</td>
<td>7.2 ± 4.3&lt;sup&gt;a&lt;/sup&gt; (4)</td>
<td>11.6 ± 8.7&lt;sup&gt;a&lt;/sup&gt; (5)</td>
</tr>
</tbody>
</table>

Drugs were administered i.p. 15 min before electrical vagus stimulation (10 min) or bethanechol (0.5 mg/kg s.c.). Parentheses indicate the number of rats used. <sup>a</sup>p < 0.01 vs. corresponding saline control.

Water-immersion-induced stress ulcers in rats. In addition, the compound abolishes preferentially the gastric secretagogue action of baclofen (centrally acting GABA-mimetics), but do not alter the action of bethanechol and histamine. There may be differences in sites of action between EPI-A and magnolol, because magnolol showed central depression in behavioural study<sup>9</sup> in contrast to EPI-A.

A γ-aminobutyrate (GABA) antagonist, bicuculline, at a dose which produced convulsions prevented baclofen-stimulated gastric acid secretion, suggesting central GABAergic control in gastric acid secretion in the rat.<sup>10,11</sup> Anti-secretory effect of EPI-A may be mediated by that control mechanism. However, the fact that 2DG- or TRH-induced acid secretion was also prevented by dopamine receptor agonists<sup>7,8</sup> suggests that central dopaminergic control mechanism also may be involved in the regulation of gastric acid secretion.

EPI-A may contribute through the CNS gastric regulatory mechanism for therapeutic effects of Pachysandra fruit in gastrointestinal disorders.

REFERENCES


4) K. Watanabe, H. Watanabe, T. Kikuchi and Z. Ryū: Central excitatory effects of pachysandra alkaloids
Epipachysamine-A on Gastric Acid Secretion


