Effects of Gabaron tea components on angiotensin I-converting enzyme activity in rat.

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Summary
Gabaron tea, a special kind of Japanese green tea rich in γ-aminobutyric acid (GABA), has a good effect on the hypertension. To show the functional mechanism of Gabaron tea, effects of GABA and some other tea components on the activity of angiotensin I –converting enzyme (ACE) in the spontaneously hypertensive rats (SHR) were investigated both in vitro and in vivo.

Results in vitro showed that GABA, Alanine, Theanine, and γ-hydroxybutyric acid had a remarkable inhibition on the ACE activity, among which GABA showed the best inhibiting ability, the ACE activity was decreased to 50% by 1mM GABA and ultimately inhibited by 30mM GABA. Results in vivo also showed that the rise of ACE activity in the SHR due to additional saline was significantly inhibited by 3% additional GABA. Suggesting that the inhibition of ACE activity by GABA is one of the major action mechanisms of Gabaron tea.

Keywords
Gabaron tea, GABA, Angiotensin I –converting enzyme, Rat

Introduction
A functional renin-angiotensin system seems to play a major role in the regulation and maintenance of both blood pressure and water as well as the sodium balance of body fluid [1-2]. Angiotensin II is known to be involved in strongly increasing the blood pressure, so substances which can inhibit the activity of angiotensin I –converting enzyme (ACE) show a good effect on the control of the hypertension [3-4]. Studies in vitro suggest that tea polyphenolic components (ECg, EGCg and theaflavin) had a notable inhibitory effect on ACE activity, and may closely related to the effect of tea on blood pressure [5]. However, information concerning the effect of Gabaron tea components on ACE activity is still scarce. To show the functional mechanism of Gabaron tea, effects of particular components of Gabaron tea, such as GABA, Alanine, Theanine, γ-hydroxybutyric acid (GHB) on the activity of angiotensin I –converting enzyme (ACE) in the spontaneously hypertensive rats (SHR) were investigated both in vitro and in vivo.

Materials and Methods
1. In vitro studies
Crude ACE was extracted from the plasma of 10 weeks male SHR rats, weighing 200-250g. The ACE inhibiting activity of GABA, Alanine, Theanine and GHB were determined by the modified method as described by Tateo Suzuki et al [5].

2. In vivo studies
Male SHR rats of 7 weeks were used. All rats were housed individually in a temperature-controlled room (23 ± 2°C, 50 ± 10%RH), illuminated between 8 a.m. and 8 p.m.
Rats were divided into four groups (n = 10 each). Group 1: Basal diet + Water; Group 2: Basal diet + 3% GABA; Group 3: 5% Saline diet + Water; Group 4: 5% Saline diet + 3% GABA. Once every week, body weight, water intake and blood pressure were recorded. After three months, all rats were anesthetized with ether. Plasma ACE was extracted and determined by the methods as described above.

**Results**

1. **Effects of Gabaron Tea Components on the Activity of Plasma ACE of rat (in vitro)**

   Compared with that of ordinary green tea, Gabaron tea contains a large amount of GABA (Usually more than 150 mg/100g) and Alanine [6]. GHB is one of the major metabolic products of administered GABA in rat [7]. To study the functional mechanism of Gabaron tea on hypertension, the ACE inhibiting activity of these characteristic components were determined in vitro. As seen in Table 1, GABA showed the best inhibitory effect on the ACE activity (89.47%), followed by Alanine (76.92%), Theanine (64%) and GHB (57.49%).

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (mM)</th>
<th>ACE activity (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>40</td>
<td>10.53</td>
<td>89.47</td>
</tr>
<tr>
<td>Alanine</td>
<td>40</td>
<td>23.08</td>
<td>76.92</td>
</tr>
<tr>
<td>Theanine</td>
<td>40</td>
<td>34</td>
<td>64.00</td>
</tr>
<tr>
<td>GHB</td>
<td>40</td>
<td>42.51</td>
<td>57.49</td>
</tr>
</tbody>
</table>

Fig 1 shows the inhibition of ACE activity by GABA at the different levels. The ACE activity could be down to 50% by 1mM GABA in the incubation solution, and ultimately inhibited by 30mM GABA. Suggesting that GABA is a good inhibitor of ACE.
2. Effect of GABA on the Plasma ACE Activity of rats (in vivo)

Fig. 2 shows the plasma ACE activity of rats treated with different diets and 3% GABA solution or tap water for three months. As seen in Fig. 2, the plasma ACE activity of rats with 5% saline diet were 1.85 nmol/min/mg, remarkably higher than that of control (1.18 nmol/min/mg). But the ACE activity of rats treated with 5% saline diet plus 3% GABA solution were lower, only 0.99 nmol/min/mg. Suggesting feeding on 3% GABA solution could significantly inhibited the rise of ACE activity in the plasma of rats due to additional saline.

![Graph showing ACE activity](image)

**Fig. 2 Effect of GABA on the ACE activity of rats loaded with saline (in vivo)**

Discussion

With regard to the functional mechanism of tea on hypertension, Yukihiki Hara et al have shown that tea polyphenolic components (ECg, EGCg and theaflavin) had a notable inhibitory effect on ACE activity. Miki Kobayashi et al have shown that the administration of theanine (γ-glutamylethylamide, one of the major components of amino acids in green tea) could also significantly decrease the blood pressure in SHR, and the direct administration of theanine into brain striatum by microinjection caused a significant increase of dopamine. Gabaron tea is a special kind of Japanese green tea, in which γ-aminobutyric acid (GABA) is accumulated abundantly by placing freshly plucked tea leaves under the anaerobic condition, and other components such as catechins and theanine show no great difference from ordinary tea. Usually tea, both green and black, contains less than 20mg% of GABA. Gabaron tea accumulates more than 150 mg% or sometimes 200 mg% of GABA. So the functional mechanism of Gabaron tea should owe to GABA. In the past experiment, the authors found that administrated GABA was rapidly incorporated into blood, GABA concentration in the plasma of rats could reached to 36.39 g/ml within one hour after the intragastrical administration of 1 mg/g body weight GABA. In this experiment, GABA has a notable inhibitory effect on plasma ACE activity in rat are demonstrated both in vitro and in vivo. Evidences supporting that the direct inhibition of ACE activity by GABA is one of the major action mechanisms of Gabaron tea.

References


