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Actions of the food components catechin, capsaicin and capsaicin glucoside on ileal contraction.

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Actions of the food components (-)-epigallocatechin gallate (EGCG, the major green tea component), capsaicin (the major pungent component) and capsaicin glucoside transformed by using the cultured plant cells of *Phytolacca Americana* on ileal contraction were investigated for evaluation of their functionality. Ileal contraction elicited by selective nerve electrical stimulation was inhibited by 100 μM EGCG and 50 μM capsaicin; however, 50 μM capsaicin glucoside did not induce any effects, possibly because of the partial change of its structure by glucosylation.

1. INTRODUCTION

Recently, effects of food components on biological function have attracted attention, and various reports about screening of the functional components in foods, development of new and high-functional materials by partial modification of the structure of known functional components, and evaluation of the potency of such components have increased. In particular, many so-called healthy foods are available with fascinating explanations for their role in enhancing good health, but their bio-availabilities are not necessarily verified scientifically. In some cases, unfortunately, unexpected health problems caused by so-called healthy foods have been reported.

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In order to evaluate the functionality and safety of food components, we have been studying the effects of food components on gut functions and the enteric nervous system, because foods are digested and absorbed from the gut. We reported the effects of catechin (the major component in green tea) on enteric (myenteric) neurons [1, 2] and the actions of capsaicin (the major pungent component) and capsaicin glucoside (pungency and water solubility were modified) on intestinal transport [3]. In this study, we investigated the actions of these food components (catechin, capsaicin and capsaicin glucoside) on intestinal contraction. We also discuss the reaction manners of these compounds on intestinal contractions by comparison with the actions of these compounds on enteric neurons.

2. MATERIALS AND METHODS

Six guinea-pigs (Hartley, male and body weight: 250–400g) obtained from Saitama Experimental Animals Supply Co. Ltd. (Saitama, Japan) and 2 rats (Wister, male and body weight: 250–400g) from Japan SLC Inc. (Shizuoka, Japan) were used for experiments for catechin at Tokyo Medical and Dental University and experiments for capsaicin and capsaicin glucoside at Chiba Institute of Science, respectively. Both animals were maintained in feeding rooms for laboratory animals and were given food

and water ad libitum. All animal experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the animal care and use committee at Tokyo Medical and Dental University and Chiba Institute of Science, respectively.

Experiments for catechin's action on gut functions were carried out by using guinea-pigs at Tokyo Medical and Dental University [1, 2] because a preparation including enteric neurons is easy to prepare from guinea-pig intestine. However, guinea-pigs are a herbivorous animal and reaction manners against various compounds differ from those in omnivorous animals like rat and mouse. Thus, to investigate the functionality and safety of food components, it might be better to use rats for such experiments. We thus used rats to study the effects of capsaicin and capsaicin glucoside on ileal contraction at Chiba Institute of Science in the same way that we studied the effects of these compounds on transmural transport [3].

The ileum was isolated from each animal after a quick head blow and bleeding from the neck. Intestinal contraction of whole ileal segments was measured by the Magnus method (Fig. 1). Ileal segments (2–3 cm) were connected with a strain force gauge (TB-611T, Nihon Kohden) and mounted in a Magnus tube filled with modified Krebs solution (NaCl 117; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ 2.5; NaH_2PO_4 1.2; $NaHCO_3$ 2.5; glucose 11.5 (mM)) saturated with 95% O_2 / 5% CO_2 and kept at 37°C.

Ileal twitch was elicited by transmural electrical stimulation with a pair of silver wires. By selecting the stimulating condition, intestinal contractions could be induced via excitation of the intramural enteric nervous system or direct stimulation of intramural smooth muscle. In the present study, an electrical stimulation was carried out with the following conditions: duration, 2–5 msec; frequency, 10 Hz; stimulating counts, 10–20 pulses. This condition became selective nerve electrical stimulation, which was confirmed by using TTX (data not shown, ileal twitch was not induced by TTX application under this stimulation). Ileal twitch was recorded on a chart recorder (Recti-Horiz, San-ei).

(-)-Epigallocatechin gallate (EGCG, Fig. 2a) known to be the largest component of green tea catechins [4], was supplied by Tokyo Food Techno Co. Ltd. (purity: >98%). Capsaicin (Fig. 2b) and capsaicin glucoside (Fig. 2c, 8-nordihydrocapsaicin β-D-glucosides transformed from 8-nordihydrocapsaicin (the sharpest pungent compound in synthetic capsaicin) using the cultured plant cells of *Phytolacca americana* [5]) were donated by H. Hamada of Okayama University of Science (purity: >99%). Sample compounds were applied to the Magnus tube (volume, 10 ml) at 10–100 μM EGCG and 50 μM capsaicin and capsaicin glucoside.

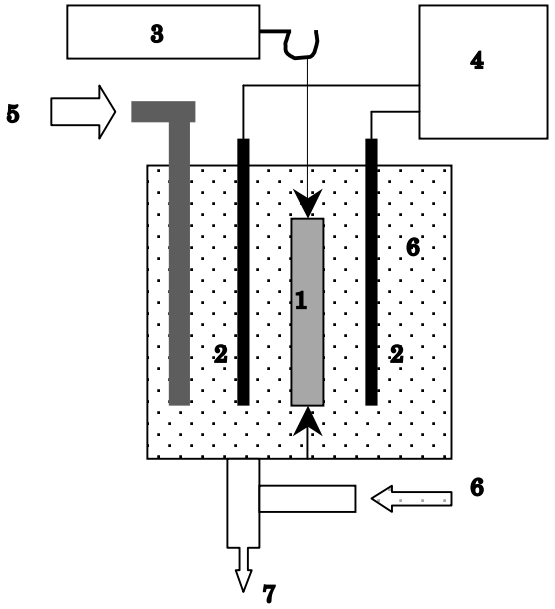

Fig. 1 Measurement of intestinal contraction by the Magnus method.
1: ileal segment, 2: silver wires, 3: strain force gauge, 4: electric stimulator, 5: 95% O_2 / 5% CO_2, 6: Krebs solution, 7: drain

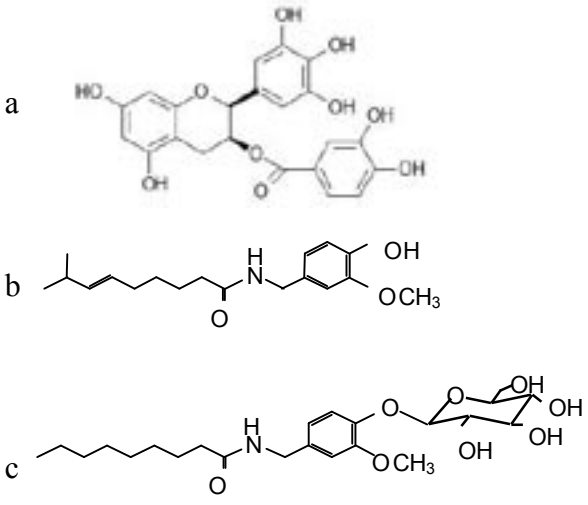

Fig. 2 Structure of (-)-epigallocatechin gallate (EGCG) (a), capsaicin (b) and 8-nordihydrocapsaicin β-D-glucosides (c).

3. RESULTS AND DISCUSSION

3.1 Actions of EGCG on intestinal contraction

The effect of EGCG on intestinal contraction in guinea-pig ileum is shown in Figure 3. The ileal twitch shown as a large signal in the figure was induced by selective nerve electrical stimulation per 30 sec. Applications of EGCG into the Magnus tube (final concentration in the tube became 10 μM and 30 μM) did not induce any change in the amplitude of ileal contractions (Fig. 3a). When the EGCG concentration became 100 μM in the Magnus tube by cumulative applications (Fig. 3b), ileal twitch decreased slightly but seemed to recover quickly. However, when EGCG was applied at 100 μM the first time, the amplitude of the ileal twitch was decreased by 60–70% of the control. This partial inhibition of ileal contraction may have occurred by the action of EGCG on the enteric nervous system or intramural smooth muscles directly.

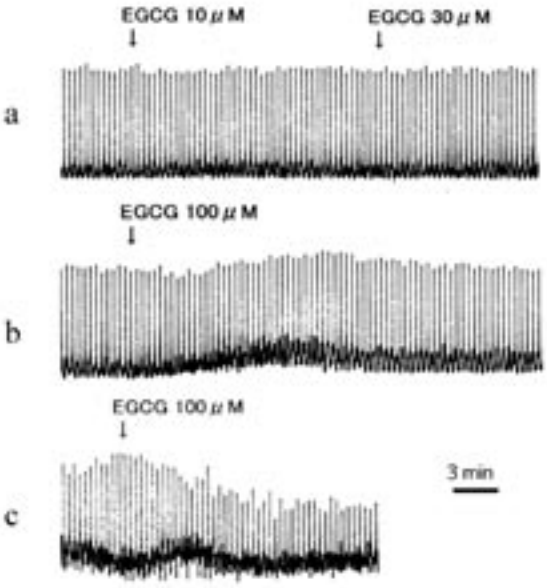

Fig. 3 Actions of EGCG on ileal contraction.
EGCG was applied cumulatively in the Magnus tube. Figures (a) and (b) are continuous. After washing the ileal preparation with Krebs solution, EGCG was applied at 100 μM EGCG the first time (c).

By changing (intensifying) the stimulating condition of electrical stimulation (duration: 30 msec, frequency: 1 Hz, stimulating count: 1 pulse), intramural smooth muscles (including, enteric nervous system too) were stimulated directly and contracted. Under this stimulating condition, ileal twitch was not affected by the application of EGCG at 100 μM (and 300 μM) (data not shown). This means that EGCG did not affect the ileal contraction itself.

As reported previously [1], EGCG induced depolarization of myenteric neurons in the guinea-pig small intestine in a concentration-dependent manner and showed marked desensitization-like characteristics (1–20 μM). The actual action concentration of EGCG against myenteric neurons in the present study might be lower than that in our previous experiment [1] because a whole segment of small intestine was used for measuring ileal contraction. It is unknown why the action at 100 μM EGCG by cumulative applications (Figs. 3a and 3b) and that after a single application (Fig. 3c) were different, and it is not yet clear whether EGCG affects other types of neurons in the enteric nervous system (sensory-, inter-, and moto- neurons). It may be that one of the reasons for the partial inhibition of ileal contraction is that a desensitization-like response may reduce the reaction against electrical stimulation.

3.2 Actions of capsaicin and capsaicin glucoside on intestinal contraction

The effects of capsaicin and capsaicin glucoside on ileal contraction in rats are shown in Fig. 4. Small jagged signals between the large signals induced by nerve stimulation per 30 sec showed repeated, automatic intestinal contraction/relaxation. These ileal contractions were partly inhibited by application of 50 μM capsaicin, but recovered after washing the ileal segments in the Magnus tube with new Krebs solution (Fig. 4a).

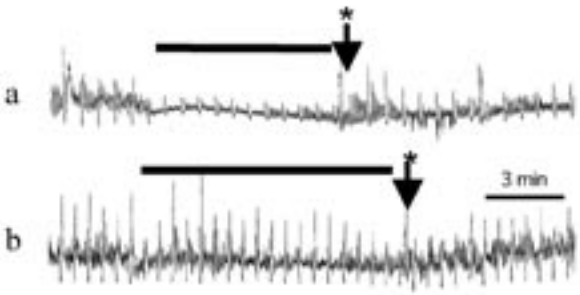

Fig. 4 Actions of capsaicin and capsaicin glucoside on ileal contraction.
Capsaicin (50 μM) inhibited ileal contraction during its application (a), but capsaicin glucoside (50 μM) did not induce any effects in ileal contraction (b). Capsaicin and capsaicin glucoside were applied during the period of the bar in the figure.
* washing of ileal segments in the Magnus tube by replacing new Krebs solution.

It was reported that capsaicin has an inhibitory effect on the twitch responses induced by field stimulation in the guinea-pig ileum [6] and evokes a marked long-lasting slow depolarization and no response to capsaicin by repeated pressure microinjection related to desensitization by capsaicin in myenteric neurons of the guinea-pig ileum [7]. Capsaicin binds with capsaicin receptor, TRPV1. It was reported that TRPV1 is widely expressed on intramural enteric ganglia and interganglionic fibers, muscle layers, the submucosal layer and mucosa throughout the lower intestinal tract [8, 9, 10]. Moreover, it is known that capsaicin has analgesic action and is used to treat neuropathic pain, which is caused by desensitization of the sensory nerve ending by capsaicin. These reports suggest that capsaicin application induces desensitization in enteric neurons and inhibition of the ileal contraction elicited by electrical stimulation. However, just after washing the ileal segments and removal of capsaicin, ileal twitch recovered quickly (Fig. 4a). Other factors for the inhibition of ileal contraction by capsaicin should be considered. Capsaicin may affect the intramural muscle layers directly and induce the inhibition of ileal contraction.

On the other hand, capsaicin glucoside did not induce any effects on ileal contraction (Fig. 4b). Binding of capsaicin glucoside with the capsaicin receptor might be stereochemically inhibited by glucosylation. That is, addition of the sugar chain on capsaicin induced structural changes and the binding manner with the receptor may be affected.

4. CONCLUSION

Because foods are digested and absorbed from the intestine, it is important and useful to evaluate food functionality and safety by investigating effects on gut functions.

EGCG showed an inhibitory effect on ileal contraction at 100 μM. After ingestion of 1.2 g of green tea in warm water, plasma samples collected at 1 hr from four human volunteers contained about 0.1–0.6 μM of EGCG [11]. These concentrations are much lower than the concentrations required for induction of an inhibitory effect on ileal contraction in the present study. Thus, drinking some cups of green tea will not cause serious problems in gut function.

Compared with the responses of capsaicin, capsaicin glucoside showed less response on ileal contraction. Combined with the result of the smaller action of capsaicin glucoside on intestinal mucosal transport [3], capsaicin glucoside might be safer for biological actions and have an advantage for use as a food ingredient.

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