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COMMUNICATIONS

Gastric H^+, K^+-ATPase inhibition by catechins

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Abstract—Five catechins, (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin and (−)-epigallocatechin gallate, inhibited gastric H^+, K^+-ATPase activity with IC50 values ranging from $1{\cdot}7 \times 10^{-4}$ to $6{\cdot}9 \times 10^{-8}$ M, with (−)-epigallocatechin gallate as the most potent inhibitor. The intensity of inhibitor activity paralleled the number of phenolic hydroxy groups in the molecule. The inhibition of the enzyme by (−)-epicatechin was competitive with respect to ATP and noncompetitive with respect to K^+. These findings suggest that the anti-secretory and anti-ulcerogenic effects of catechins previously reported, are due to their inhibitory activity on gastric H^+, K^+-ATPase.

Catechins are bioflavonoids and are distributed mainly in higher woody plants. (+)-Catechin has been reported to be a specific inhibitor of histidine decarboxylase, which plays a role in the formation of histamine from histidine (Lorenz et al 1973, 1975). The catechin derivative, meciadanol (*O*-methyl-3-(+)-catechin) also inhibits histidine decarboxylase (Wendt et al 1980). Catechins have been shown to be effective in the inhibition of gastric acid secretion and experimental ulcers (Reimann et al 1977; Parmar & Ghosh 1981; Albinus et al 1983; Parmar & Hennings 1984; Jayaraj et al 1988). Since histamine plays an important role as a mediator in acid secretion, and histamine H_2-receptor antagonists such as cimetidine, ranitidine and famotidine are highly effective in reducing acid secretion, the anti-ulcerogenic and anti-secretory activities of catechins have been considered to be closely related to histidine decarboxylase inhibition (Reimann et al 1977; Albinus et al 1983; Parmar & Hennings 1984). Gastric H^+, K^+-ATPase is also an important enzyme involved in acid secretion. This enzyme catalyses H^+ transport at the expense of ATP hydrolysis in the final step of gastric acid secretion (Sachs et al 1976; Forte et al 1980). Therefore, the inhibition of the enzyme leads to the reduction of acid secretion. A selective H^+,K^+-ATPase inhibitor, omeprazole (Fellenius et al 1981), which shows a potent anti-secretory activity, is clinically used for therapy of peptic ulcers (Gustavsson et al 1983; Lind et al 1983). We have shown that naturally occurring compounds including salvianolic acid A (Murakami et al 1991a), chalcones (Murakami et al 1990b, 1991b) and ellagic acid (Murakami et al 1991a) are inhibitors of gastric H^+, K^+-ATPase. In this paper, we report the inhibitory effect of catechins on H^+, K^+-ATPase.

Materials and methods

Materials. (+)-Catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin and (−)-epigallocatechin gallate were purchased from Funakoshi Co. Ltd, Tokyo, Japan. ATP disodium salt was purchased from Sigma Chemical Co. (St Louis, MO, USA) and converted to Tris salt in our laboratory. All other chemicals were of the highest reagent grade available. Fresh hog stomachs were purchased from the local abbatoir.

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Preparation of gastric H^+, K^+-ATPase. Stomachs from freshly slaughtered hogs were flushed with tap water and were cleaned with a paper towel, then the fundic mucosal layer was removed from the underlying tissue with a surgical blade. Gastric microsomal vesicles containing H^+, K^+-ATPase were prepared by density gradient centrifugation (Saccomani et al 1977). The purified vesicles were collected and lyophilized to make them freely permeable to cations, and stored at −80°C. Protein was determined by the Lowry method, using bovine serum albumin as the standard (Lowry et al 1951).

Assay of H^+, K^+-ATPase. The assay medium consisted of 2 mM $MgCl_2$, 2 mM Tris-ATP, 40 mM Tris-HCl pH 7·4 and 5 μg membrane protein, with or without 20 mM KCl in a total volume of 1 mL. The medium was incubated for 20 min at 37°C. The reaction was terminated by the addition of 1 mL ice-cold trichloroacetic acid (10%). The inorganic phosphate derived from ATP was measured according to Fiske & Subbarow (1925). Compounds tested were dissolved in dimethylsulphoxide. The concentration in the reaction mixture did not exceed 1·0% and this concentration did not influence the enzyme activity. Results are expressed as mean ± s.e. of duplicate experiments (n = 4).

Results and discussion

There have been several reports concerning the anti-secretory and anti-ulcerogenic effect of catechins (Reimann et al 1977; Parmar & Ghosh 1981; Albinus et al 1983; Parmar & Hennings 1984; Jayaraj et al 1988). We investigated the effect of five catechins, (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin and (−)-epigallocatechin gallate (Fig. 1) on gastric H^+, K^+-ATPase activity to determine the mechanism by which catechins inhibit acid secretion. All these catechins inhibited H^+, K^+-ATPase from hog gastric mucosa (Fig. 2). The IC50 values were as follows: (+)-catechin $1{\cdot}7 \times 10^{-4}$ M, (−)-epicatechin $4{\cdot}7 \times 10^{-5}$ M, (−)-epigallocatechin $9{\cdot}3 \times 10^{-5}$ M, (−)-epicatechin gallate $1{\cdot}1 \times 10^{-7}$ M, (−)-epigallocatechin gallate $6{\cdot}9 \times 10^{-8}$ M. The present findings show that catechins inhibit gastric H^+, K^+-ATPase.

To elucidate the mechanism by which catechins inhibit H^+, K^+-ATPase activity, kinetic studies were carried out using (−)-epicatechin. Measurement of the inhibition of H^+, K^+-ATPase activity at various ATP concentrations, and double reciprocal plot analysis suggested a competitive interaction between ATP and the compound (Fig. 3). Changes in apparent K_m from 1·06 to 1·35, 1·75 and 2·13 mM in the presence of 20, 40 and 60 μM (−)-epicatechin, respectively, were observed. The calculated K_i value was 49 μM. On the other hand, noncompetitive inhibition was found for K^+ (Fig. 4). The apparent V_{max} values were decreased from 181 to 120, 91 and 70 μmol h^{-1} (mg protein)$^{-1}$ in the presence of 20, 40 and 60 μM (−)-epicatechin, respectively. The calculated K_i value was 35 μM. In the H^+, K^+-ATPase system, the enzyme binds to ATP on the cytosolic ATP site to form a phosphoenzyme in the presence of Mg^{2+}. The phosphoenzyme is then dephosphorylated by luminally bound K^+ (Wallmark & Mardh 1979). The present kinetic studies suggest that (−)-

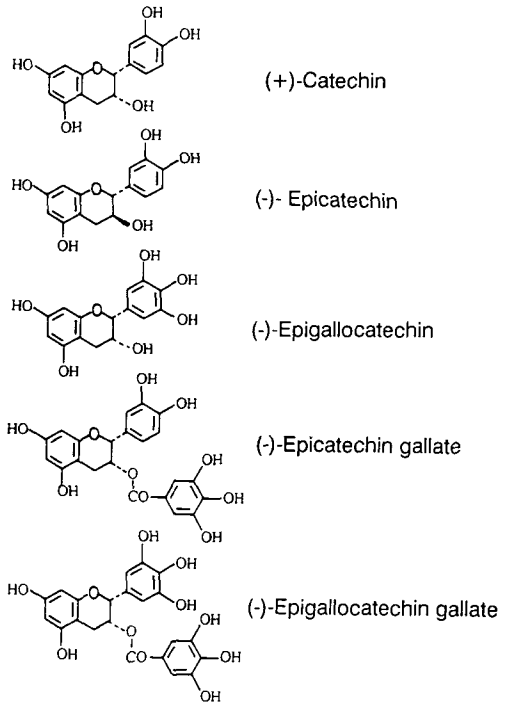

FIG. 1. Chemical structures of catechins investigated.

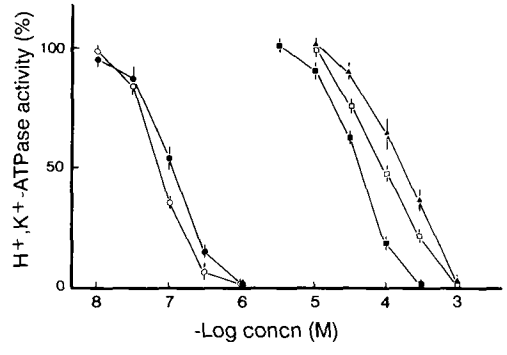

FIG. 2. Effect of (+)-catechin (▲), (−)-epicatechin (■), (−)-epigallocatechin (□), (−)-epicatechin gallate (●) and (−)-epigallocatechin gallate (○) on gastric H^+, K^+-ATPase from hog gastric mucosa. Each point represents the average of duplicate experiments (n=4). Vertical bars indicate s.e. Control 100% H^+, K^+-ATPase activity was 157 μmol h^{-1} (mg protein)$^{-1}$.

epicatechin competes with ATP and inhibits the formation of phosphoenzymes.

We have previously shown that naturally occurring phenolic compounds, salvianolic acid A (Murakami et al 1990a) and ellagic acid (Murakami et al 1991a) are inhibitors of gastric H^+, K^+-ATPase. The inhibition of H^+, K^+-ATPase by these compounds occurs similarly to that by (−)-epicatechin, competitive with regard to ATP and noncompetitive with respect to K^+. The previous study also indicated that phenolic hydroxy groups are important in the inhibition of H^+, K^+-ATPase (Murakami et al 1990a). In the present study, the intensity of the inhibitory activity of the catechins depended on the number of hydroxy groups in the molecule. Among the catechins tested, (−)-epigallocatechin gallate, which has 8 hydroxy groups, had the strongest inhibitory activity, while (+)-catechin, and (−)-

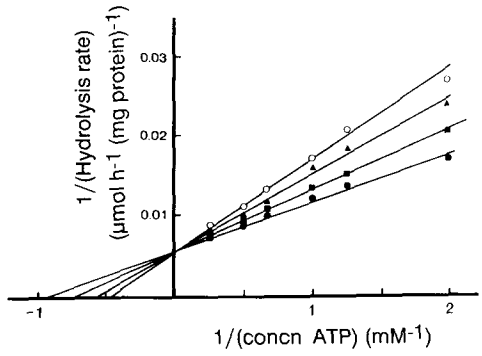

FIG. 3. Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase vs concentrations of ATP in the presence of 0 (●), 20 (■), 40 (▲) and 60 μM (○) (−)-epicatechin. Each point represents the average of duplicate experiments (n=4).

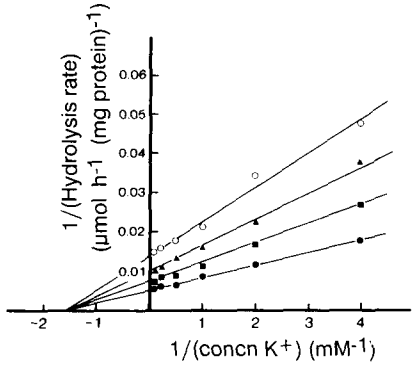

FIG. 4. Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase vs concentrations of KCl in the presence of 0 (●), 20 (■), 40 (▲) and 60 μM (○) (−)-epicatechin. Each point represents the average of duplicate experiments (n=4).

epicatechin, with 5 hydroxy groups, had weaker inhibitory activity. This also shows the importance of the interaction between the phenolic hydroxy groups and H^+, K^+-ATPase. Moreover, (+)-catechin and (−)-epicatechin, which have the same chemical structure, but a different steric position of the 2- and 3-substituent, showed different inhibitory activity; the 50% inhibition values were $1{\cdot}7 \times 10^{-4}$ ((+)-catechin) and $4{\cdot}2 \times 10^{-5}$ M ((−)-epicatechin). This indicates the importance of a steric interaction at this position with the H^+, K^+-ATPase. The anti-secretory and anti-ulcerogenic effects of catechins have been considered to be closely related to their inhibitory action on histidine decarboxylase (Reimann et al 1977; Parmar & Ghosh 1981; Albinus et al 1983; Parmar & Hennings 1984). However, the present findings suggest that the anti-secretory and anti-ulcerogenic effects of the catechins may in part be due to the inhibition of H^+, K^+-ATPase, since H^+, K^+-ATPase plays an important role in acid secretion.

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