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INHIBITORY EFFECT OF TANNIC ACID ON GASTRIC H^+, K^+-ATPASE

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ABSTRACT.—The effect of tannic acid on gastric H^+, K^+-ATPase was studied. Tannic acid dose-dependently inhibited pig gastric H^+, K^+-ATPase activity with an IC_{50} value of 2.9×10^{-8} M. Tannic acid also inhibited K^+-stimulated p-nitrophenyl phosphatase (K^+-pNPPase) activity, which is found in gastric H^+, K^+-ATPase preparations, as well as H^+, K^+-ATPase activity, with an IC_{50} value of 4.1×10^{-7} M. Kinetic studies showed that the inhibition of H^+, K^+-ATPase activity by tannic acid was competitive with respect to ATP and noncompetitive with respect to K^+. These results show that tannic acid is a potent inhibitor of gastric H^+, K^+-ATPase; this may be related to its anti-secretory and anti-ulcerogenic effects.

Tannins, including tannic acid, are common constituents of plants used in oriental medicine. They have a variety of biological and pharmacological activities (1–5); among these, anti-secretory and anti-ulcerogenic effects have been reported. A crude extract from *Linderae ramus*, of which the main constituent is tannin, has been shown to be effective in various kinds of experimental ulcer models in rats (6). Tannic acid inhibits gastric acid secretion in pylorus-ligated rats and mice (7,8); it is also effective in acute and chronic experimental ulcer models (8,9). However, the mechanisms of anti-secretory and anti-ulcerogenic actions of tannic acid are not known. Gastric H^+, K^+-ATPase is a membrane enzyme which catalyzes H^+ transport from parietal cells into the gastric cavity in response to ATP hydrolysis (10,11). This is an important final step in gastric secretion. In this paper, we report the effect of tannic acid on gastric H^+, K^+-ATPase.

EXPERIMENTAL

MATERIALS.—Tannic acid was purchased from Sigma Chemical, St. Louis. All other chemicals were of the highest purity commercially available. Fresh pig stomachs were purchased from the local slaughterhouse.

GASTRIC H^+, K^+-ATPASE PREPARATION.— Stomachs from freshly slaughtered pigs were flushed with tap H_2O and cleaned with paper towels, and the mucosal layer was removed from the underlying tissue by using a surgical knife. The membrane vesicle fraction containing H^+, K^+-ATPase was prepared by Ficoll-sucrose density gradient centrifugation, as described previously (12). The membrane fraction fractionated above the Ficoll interface was used for the experiment. The vesicles obtained were collected and lyophilized to render them freely permeable to cations and were stored at $-80°$ until use.

H^+, K^+-ATPase ASSAY.—Membrane protein (5 μg) was incubated in 1 ml of 40 mM Tris-HCl buffer (pH 7.4) containing 2 mM $MgCl_2$ and 2 mM ATP Tris salt, with or without 20 mM KCl, for 20 min at 37°. The reaction was terminated by the addition of 1 ml of 10% ice-cold trichloroacetic acid, and an assay for inorganic phosphate was performed according to the method of Fiske and Subbarow (13). Tannic acid was dissolved in distilled H_2O. H^+, K^+-ATPase activity was calculated by subtracting basal Mg^{2+}-stimulated activity from enzyme activity in the presence of K^+ and Mg^{2+}. Protein content was measured by the method of Lowry *et al.* (14), using bovine serum albumin as a standard.

K^+-pNPPase ASSAY.—The assay medium contained, in a total volume of 1 ml, 40 mM Tris-HCl buffer, 5 mM $MgCl_2$, 5 mM p-nitrophenyl phosphate (pH 7.4), and 5 μg membrane protein, with or without 20 mM KCl. After 20 min incubation at 37°, the reaction was terminated by the addition of 1 ml 1 M NaOH. The absorbance of the reaction medium was measured at 410 nm (15).

RESULTS AND DISCUSSION

The data from this experiment show that tannic acid is a potent inhibitor of gastric H^+, K^+-ATPase. Tannic acid dose-dependently inhibited gastric H^+, K^+-ATPase activity, with an IC_{50} value of 2.9×10^{-8} M (Figure 1). This gastric H^+, K^+-ATPase preparation contains K^+-pNPPase, which has been
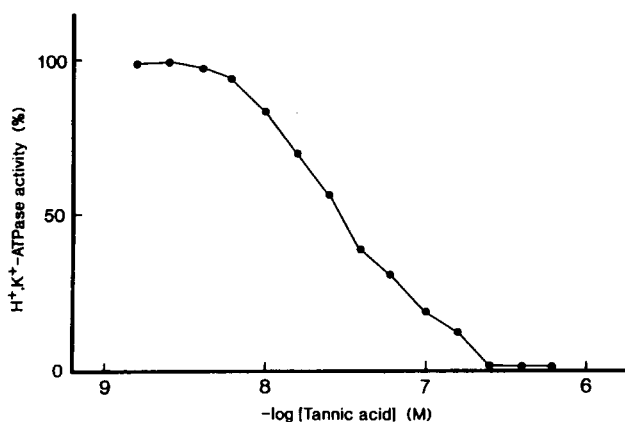
FIGURE 1.   Effect of tannic acid on H^+,K^+-ATPase from pig gas-
tric mucosa. Microsome membranes (5 μg protein)
were incubated in 40 mM Tris-HCl buffer containing 2
mM $MgCl_2$, 20 mM KCl, and 2.5 mM Tris-ATP, in a
total volume of 1 ml, for 20 min at 37°. The reaction
was stopped by the addition of tricholoroacetic acid
(final concentration 5%), and liberated inorganic phos-
pate was determined. Each point represents the mean of
duplicate experiments ($n = 4$).

regarded as the dephosphorylation step
of H^+,K^+-ATPase (16). Tannic acid
also inhibited pNPPase activity in a
dose-dependent manner (Figure 2). The
IC_{50} value was 4.1×10^{-7} M. To eluci-

date the mechanism by which tannic
acid inhibits H^+,K^+-ATPase, the in-
hibition of its activity was measured as a
function of ATP and K^+ concentration.
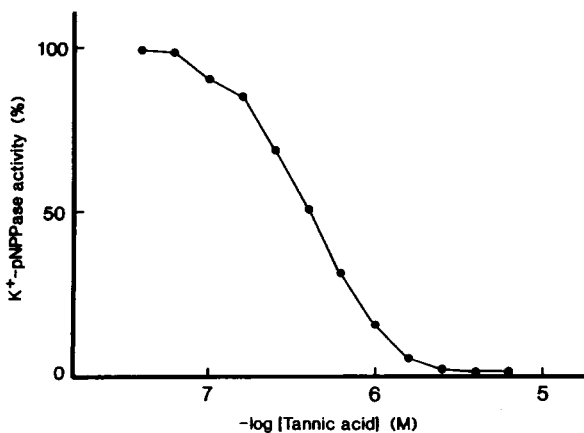Double reciprocal plots showed the in-

FIGURE 2.   Effect Nof tannic acid on K^+-pNPPase from pig
gastric mucosa. Microsome membranes (5 μg pro-
tein) were incubated in 40 mM Tris-HCl buffer
containing 5 mM $MgCl_2$, 20 mM KCl, and 5 mM
p-nitrophenyl phosphate in a total volume of 1 ml,
for 20 min at 37°. The reaction was stopped by the
addition of 1 ml 1 M NaOH, and absorbance was
measured at 410 nm. Each point represents the
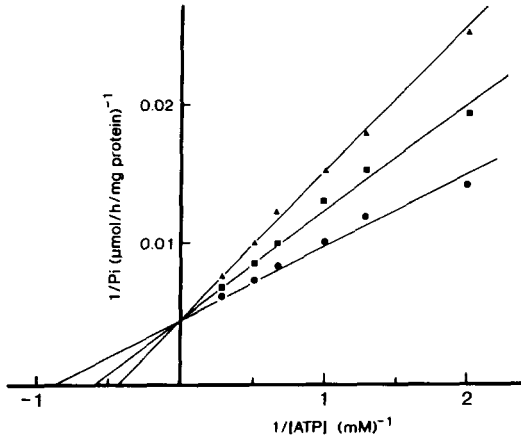mean of duplicate experiments ($n = 4$).

FIGURE 3. Double reciprocal plots of the rates of ATP hydrolysis by H^+,K^+-ATPase versus concentration of ATP in the presence of 0 (●), 2.5×10^{-8} M (■), and 5.0×10^{-8} M (▲) tannic acid. Each point represents the mean of duplicate experiments ($n = 4$).

hibition of gastric H^+,K^+-ATPase by tannic acid to be competitive with respect to ATP (Figure 3) and noncompetitive with respect to K^+ (Figure 4). The ATP hydrolytic site of H^+,K^+-ATPase is located on the cytosolic face, which is opposite the K^+ site (17). In the presence of Mg^{2+}, the enzyme binds to ATP to form a phosphoenzyme, and it is then dephosphorylated by luminal bound K^+. It is suggested from the present results that tannic acid may compete with ATP and thereby inhibit the formation of the intermediate phosphoenzyme. We have shown in a previous report that a naturally occurring polyphenol, ellagic acid, inhibits gastric H^+,K^+-ATPase (18). The H^+,K^+-ATP-
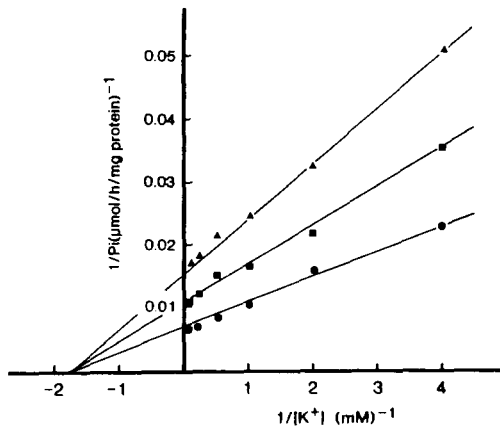

FIGURE 4. Double reciprocal plots of the rates of ATP hydrolysis by H^+,K^+-ATPase versus concentration of KCl in the presence of 0 (●), 2.5×10^{-8} M (■), and 5.0×10^{-8} M (▲) tannic acid. Each point represents the mean of duplicate experiments ($n = 4$).

ase inhibition pattern induced by el-
lagic acid was the same as that induced
by tannic acid, competitive with respect
to ATP and noncompetitive with respect
to K^+. However, the inhibition of gas-
tric H^+,K^+-ATPase by tannic acid was
about ten times more potent than that
by ellagic acid, which has an IC_{50} value
of 2.1×10^{-7} M. Tannic acid has more
phenolic hydroxy groups than ellagic
acid. The difference in magnitude of en-
zyme inhibition is probably due to the
number of hydroxy groups in the
molecule, because phenolic hydroxy
groups have some interactions with the
ATP site of gastric H^+,K^+-ATPase, as
suggested in previous studies (18,19).

Since gastric H^+,K^+-ATPase plays
an important role in the secretion of acid
from the parietal cells of the gastric wall
into the lumen, inhibition of this en-
zyme results in the reduction of acid se-
cretion. A synthetic anti-ulcer agent,
omeprazole, an inhibitor of gastric
H^+,K^+-ATPase, has been used for the
therapy of peptic ulcer (20). It is con-
ceivable from the present results that the
anti-secretory and anti-ulcerogenic ef-
fects of tannic acid are related in part to
its inhibitory effect on gastric H^+,K^+-
ATPase.

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