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A PUNGENT INGREDIENT OF MUSTARD, ALLYLISOTHIOCYANATE, INHIBITS (H^++K^+)-ATPASE

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Allylisothiocyanate (ANCS) is shown to inhibit (H^++K^+)-ATPase isolated from the parietal cell of hog gastric mucosa. The ATPase is the proton pump in the secretory membrane of the parietal cell and is an essential component of the acid secretory mechanism. Furthermore, ANCS suppresses acid secretion by bullfrog gastric mucosa in vitro. We suggest that one aspect of the stomachic function produced by ANCS-including foods is the suppressive effect of ANCS on the acid secretory mechanism.

ANCS is a pungent ingredient of mustard, horseradish, Wasabia japonica, etc. When ANCS-including food is taken by human, the pungent material is traditionally supposed to induce a stomachic action although no biochemical and physiological studies on gastric secretion has been reported. In this study we will show that ANCS inhibits (H^++K^+)-ATPase which is an electroneutral H^+/K^+ exchange pump and involved in the terminal steps of gastric secretory process (1-4).

MATERIALS AND METHODS

Gastric vesicles —— Hog gastric vesicles enriched in the gastric (H^++K^+)- ATPase were collected by ultracentrifugation in a discontinuous gradient at the interface of 0.25 M sucrose, 7 % Ficoll + 0.25 M sucrose layers as described elsewhere (3,5).

Enzyme assay ——(H^++K^+)-ATPase activity was measured in a 1 ml solution containing 50 - 80 μg protein, 3 mM $MgCl_2$, 3 mM ATP, 15 mM KCl and 40 mM Tris/acetate (pH 7.4) as described elsewhere (5). The reaction was done at 37°C for 10 min.

Proton transport activity by vesicles —— The vesicles were preincubated for 2 hr at room temperature with 150 mM KCl, 2 mM $MgCl_2$, and 5 mM glycylglycine (pH 6.11). At zero time, 0.3 mM MgATP was added to the vesicle solution (0.1 mg of protein/ml) and the time course of the change in external pH which showed a maximum was recorded as described elsewhere (6).

Salt conductances of the vesicle membrane from light scattering —— The relative salt conductances of the vesicle membrane were assessed by monitoring

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Abbreviations; ANCS, allylisothiocyanate. SITS, 4-acetoamide-4'-isocyanostilbene-2,2'-disulfonate

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changes in light scattering of vesicle preparations produced by salt gradients across the membrane as described elsewhere (5,6).

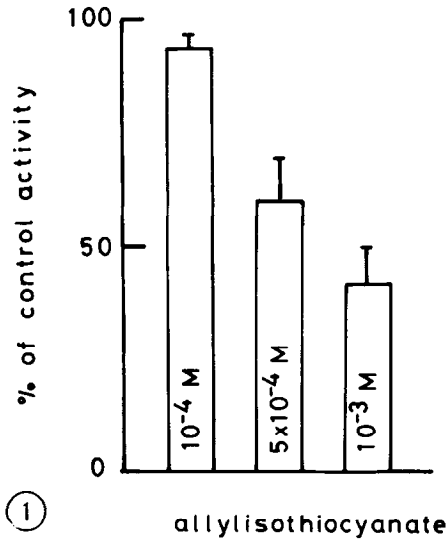
Acid secretion by bullfrog gastric mucosa ——The mucosa was set between Ussing chambers and the rate of HCl secretion at 25°C was determined from a pH stat method (pH 4.6) as described elsewhere (7,8). The serosal solution included (in mM) 5 K^+, 105 Na^+, 1 Mg^{2+}, 2 Ca^{2+}, 97 Cl^-, 18 HCO_3^-, 1 $H_2PO_4^-$, 11 glucose, and 0.1 mM histamine base. The mucosal solution included 120 mM NaCl and different concentrations of ANCS (indicated in the figure). Both solutions were gassed with 95 % O_2 + 5 % CO_2 and were exchanged for new solutions every 15 min.

RESULTS AND DISCUSSION

Preincubation of vesicles with 1 mM ANCS for 30 min at 25°C affects neither the enzyme activity nor the H^+- transporting activity of the ATPase. But, a prolonged preincubation with ANCS (24 hr at 4 ° C) decreases the enzyme activity (Fig. 1). Furthermore, the prolonged treatment with 1 mM ANCS decreased the maximum proton transport in 150 mM KCl equilibrated vesicles to a level, 53 $\pm$ 10 % of control (mean $\pm$ s.e. for 3 experiments).

We can consider two possibilities for the effects of prolonged incubation with ANCS: the first is that the ANCS reaction on the external surface of the ATPase is very slow, and the second is that the ANCS-inhibitory site is located in the vesicle interior.

To specify the inhibitory mechanism of ANCS, we examined recent our findings by using ANCS. That is, a) closed anion channels in the gastric vesicles can be reopened by S-S cross-linking produced by treatment with Cu^{2+} + o-phenanthroline, resulting in the increase of the conductance of salts such as KCl and NaCl across the gastric vesicle membrane , b) pretreatment with an anion channel inhibitor, SITS, prevents the formation of S-S cross-links(5). The open-close of anion channel in vivo has been suggested to engage in the regulation of acid secretory mechanism (5,9,10). The incubation of vesicles with 1 mM ANCS for 30 min at room temperature did not affect the salt conductance. However, the incubation with ANCS antagonizes the effect of the subsequent incubation with Cu^{2+} (5 μM) + o-phenanthroline (10 μM) (30 min) as shown in Fig. 2. This indicates that SH groups at the external site of the anion channel are modified by the 30 min treatment with ANCS as well as in the case of SITS. The reaction would be



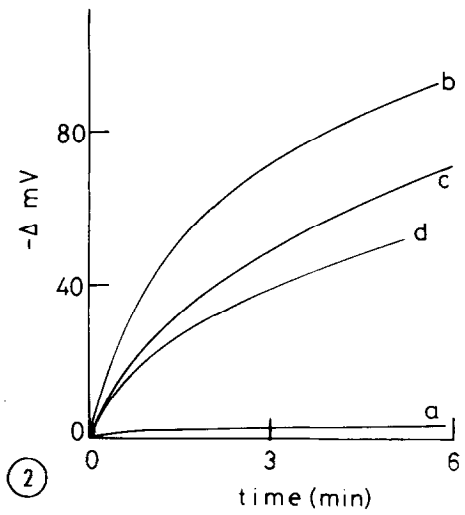


Fig. 1 Activity of $(H^+ + K^+)$-ATPase preincubated with different concentrations of allylisothiocyanate for 24 hr at 4° C. The results are mean ± s.e. of 3-4 observations. The control activity was 17.9 ± 0.4 u moles Pi/mg of protein/hr at 37° C.

Fig. 2 Effects of successive ANCS and Cu^{2+}-o- phenanthroline incubations on KCl entry into the gastric vesicles. Gastric vesicles suspended in 0.25 M sucrose solution (0.1 mg of protein/ml) were preincubated with ANCS for 30 min at 25° C. The ANCS concentration was 0 for curve b, 0.1 mM for curve c, and 1 mM for curve d. Then, the vesicles were preincubated with 5 μM Cu^{2+} + 10 μM o-phenanthroline for 30 min at 25° C (curves, b- d). The preincubated vesicles or the control vesicles (not treated with ANCS and Cu^{2+} + o-phenanthroline, curve a) were mixed with 300 mM KCl solution in a 1:1 volume ratio by using a Durrum stopped flow system. Upon mixing of the two solutions, the intensity of 90° light scattering from the mixed solution changed in two phases. The initial rapid phase is due to shrinkage of the vesicles produced by water efflux (not shown). The second slow phase which is shown here is due to reswelling of vesicles produced by solute entry accompanied with water influx[5, 6]. In this figure, the larger slope means the larger KCl conductance of the vesicle membrane. A typical experiment out of 4 experiments is shown.

$$\text{Protein-SH} + CH_2{=}CH{-}CH_2{-}N{=}C{=}S \longrightarrow \text{Protein-S-}\overset{\overset{S}{\|}}{C}\text{-NH-}CH_2{-}CH{=}CH_2 \ ,$$

as previously suggested from studies on the inhibition of papain by ANCS (11). However, administration of ANCS from the vesicle exterior does not affect the activity of the ATPase. These results argue in favor of the second possibility.

Another supporting fact for the second possibility was obtained from studies on acid secretion by bullfrog gastric mucosa in vitro. Since the luminal (mucosal) side of the mucosa is considered to correspond to the vesicle interior (12), ANCS was added to the mucosal solution. The addition

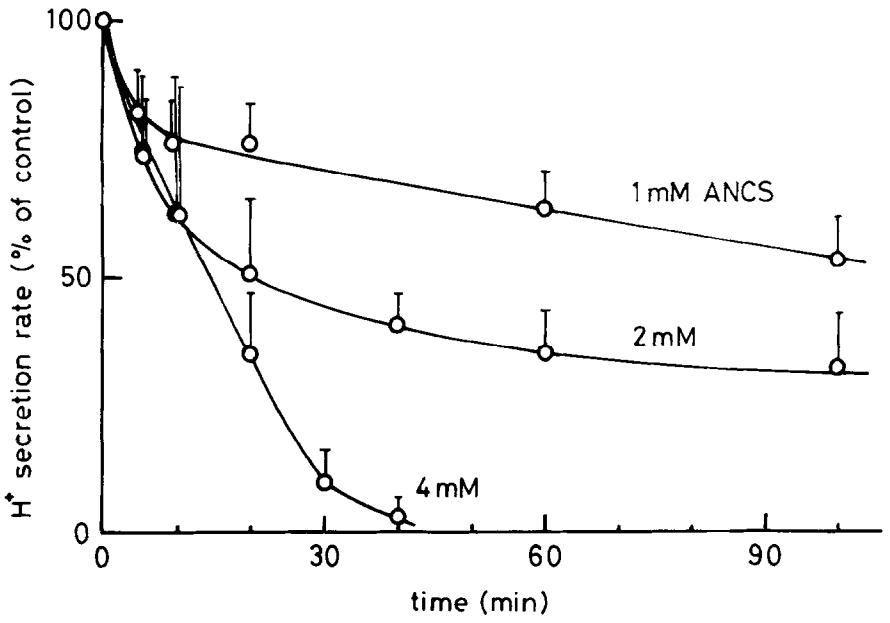


Fig. 3 Effects of ANCS on gastric acid secretion by bullfrog gastric mucosa in vitro at 25° C. The results are mean ± s.e. of 4 experiments. The control rate (no ANCS in solutions) was 5.9 ± 0.2 $\mu Eq/cm^2/hr$ and was constant for 6-12 hrs.

suppresses acid secretion dose-dependently as shown in Fig. 3. These results suggest that ANCS in stomach can inhibit the $(H^+ + K^+)$- ATPase. When ANCS-including food is eaten and the ANCS concentration attains the physiological level, at least at *loci* of stomach, it will work toward suppression of acid secretion.

If the stomachic action is considered to produce the balanced secretion (e.g., too much is as bad as too little.), some stomachic food is implied to include ingredients to stimulate acid secretion or/and ingredients to suppress it. Present findings afford a biochemical and physiological explanation for the possible mild stomachic action of ANCS-including food.

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