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The perparation of (Na^+-K^+)-dependent ATPase from human gastric mucosa

The (Na^+-K^+)-dependent ATPase activity present in membrane fractions prepared from various tissues is functionally related to the active transport of Na^+ and K^+ across the cell membrane[1-6]. This ion transport system is the biochemical basis for the electrical membrane potencial in most cells. In the secretory cells of the stomach, however, the electrical activity is apparently maintained, at least in part, by the active transport of Cl^-.

An (Na^+-K^+)-dependent ATPase from cat gastric mucosa was reported by BONTING *et al.*[8], but subsequent efforts to prepare (Na^+-K^+)-dependent ATPase from gastric mucosa have failed[7,9-11]. The gastric "membrane" or microsomal fractions described by these workers had an ATPase activity which was stimulated by Cl^- and bicarbonate and was neither stimulated by Na^+ and K^+ nor inhibited by ouabain; they have proposed an ATP-dependent Cl^- pump in these cells[7,11].

In the following we would like to present data showing that a "membrane fraction" with a typical (Na^+-K^+)-stimulated ATPase activity can be prepared also from the human gastric mucosa.

The gastric mucosa was obtained from resected human stomachs. The specimens were rinsed in ice-cold 0.32 M sucrose, minced with scissors and homogenized in 9 vol. of 0.32 M sucrose containing 0.5 mM EDTA. The homogenate was then diluted with an equal amount of the NaI solution described by NAKAO *et al.*[12], giving final concentrations of 2.0 M NaI, 2.5 mM cysteine, 5.0 mM $MgCl_2$ and 4.0 mM ATP. The mixture was rehomogenized, incubated 10 min at 0° and then centrifuged at 20000 × *g* for 20 min. The supernatant was diluted with ice-cold water to 0.6 M NaI, rehomogenized and centrifuged again at 20000 × *g* for 20 min. The supernatant was discarded, and the precipitate was washed twice by resuspension and rehomogenization in ice-cold water followed by centrifugation at 20000 × *g* for 20 min. Finally the precipitate was suspended in cold water to the volume of the first sucrose homogenate. This suspension ("membrane material") was frozen and stored at —30° for 1 day to 4 weeks before ATPase activities were tested.

Prior to ATPase assay, the preparations were thawed and rehomogenized. The ATPase activity of membrane fraction was measured by liberation of P_i in an incubation system containing 0.25 ml of membrane suspension, 1.0 ml of 100 mM Tris–histidine buffer (pH 7.1), 0.5 ml of 10 mM Tris–ATP (pH 7.1), 0.5 ml of 10 mM $MgCl_2$ and 0.25 ml of either water or salt solution (0.33 M KCl and 0.80 M NaCl). The ouabain was dissolved in the same salt solution. The tubes were incubated at 40° for 60 min, the reaction was stopped by adding ice-cold 8 % perchloric acid and the precipitate was removed by filtration. The P_i was measured by a modification of the method of FISKE AND SUBBAROW[13].

The results are presented in Table I.

Similar results were also obtained when rat stomachs were used. In this case, the (Na^+-K^+)-dependent part of the ATPase activity was studied separately in the glandular (pyloric) part (which is responsible for the secretory function) and membranous (rumenal) part of the stomach, and the activity (per mg tissue) in the secretory part was at least as high as, or higher than, that found in similar preparations for the membranous part[14].

TABLE I

ATPase ACTIVITY OF MEMBRANE FRACTION FROM HUMAN GASTRIC MUCOSA

Each value represents the mean of 8 experiments ± S.E.

Preparation	P_i *liberated* (*μmoles/mg dried "membrane material" per h*)	*Percent*	*P value*
Mg^{2+}-dependent only (basic)	0.842 ± 0.065	61 ± 5	<0.001
Total (Mg^{2+}-dependent and (Na^+-K^+)-dependent)	1.370 ± 0.022	100 ± 2	—
(Na^+-K^+)-dependent only	0.528 ± 0.068	38 ± 4	<0.001
Ouabain (1 mM)	0.303 ± 0.056	22 ± 4	<0.001

Efforts to prepare fractions with a (Na^+-K^+)-dependent ATPase by differential centrifugation of sucrose homogenates were unsuccessful. The fractions obtained by this procedure had ATPase activities which were unaffected or inhibited by Na^+ and K^+ and unaffected or stimulated by ouabain.

The results presented above have shown that the (Na^+-K^+)-dependent ATPase could be prepared from human gastric mucosa using an NaI treatment. In untreated preparations, the (Na^+-K^+)-dependent ATPase probably is masked by other ATP-hydrolyzing enzyme systems some of which, like the ATPase of myosin B, might be stimulated by ouabain and inhibited by monovalent cations[15].

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