Mg^{2+}-HCO_3^--ATPase and carbonic anhydrase in rat intestinal mucosa

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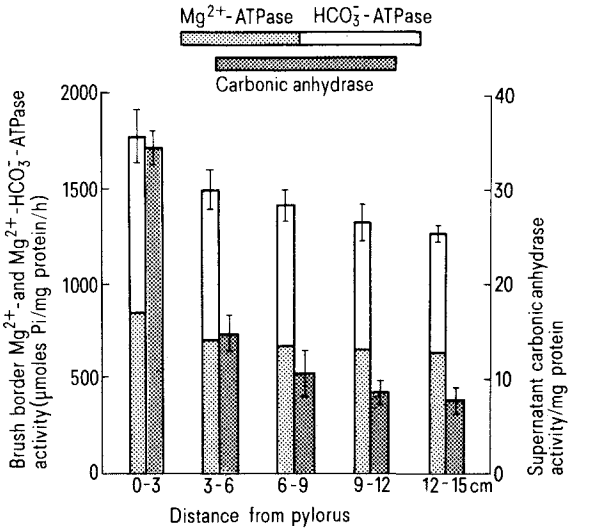
Summary. Mg^{2+}-dependent and HCO_3^--stimulated ATPase activity was highest in the brush border (microvilli) of rat duodenal mucosa compared with that of the other gastrointestinal mucosa. This ATPase may be useful to neutralize the gastric acid in the duodenal lumen. Carbonic anhydrase seems to accomplish a subsidiary role in the above reaction.

Although it is well known that the gastric acid moves into the duodenal lumen and is neutralized, the mechanism of this phenomenon has not been established. Several investigators have suggested that this neutralization is due to the reaction of gastric acid with $NaHCO_3$ derived from the bile or pancreatic juice. On the other hand, Harmon et al.[1] offered the hypothesis that the duodenal mucosa itself has the ability to neutralize the gastric acid during the following bicarbonate neutralization reaction, $NaHCO_3 + HCl \rightarrow NaCl + CO_2 + H_2O$. Our assumption is that the $NaHCO_3$ in the above reaction may be brought from the duodenal mucosa. However, biochemical and physiological properties of the duodenal mucosa itself have not yet been established. Mg^{2+}-HCO_3^--ATPase has recently been assumed to be related to the membrane transport of H^+, Cl^-, and HCO_3^- in rat small intestinal mucosa[2] and renal tubule[3]. In the present experiment, we examined the distribution of Mg^{2+}-HCO_3^--ATPase and carbonic anhydrase activities in various gastrointestinal tracts, especially duodenal mucosa, of rats to clarify whether the duodenal mucosa has specific properties compared with those of the other segments.

Methods. Male Wistar rats weighing 300–350 g were used. Animals were fasted for about 20 h with access to tap water ad libitum prior to sacrifice. Under pentobarbital anesthesia (30 mg/kg, i.p.), the abdomen was opened and all organs were perfused via the portal vein and aorta with cold heparin-saline. The small intestine, from the pylorus to the end of the ileum, was excised and cut into 7 segments, each approximately 15 cm in length. The lumen of the stomach, duodenum (segment 1), jejunum (segment 3), ileum (segment 6) and colon with rectum was flushed with cold saline. The mucosa of these organs was scraped off with a glass slide and samples from 3 rats were combined and used as 1 sample. The mucosa was homogenized with a glass-teflon homogenizer in 50 mM mannitol-2 mM Tris-HCl buffer (pH 7.1 at 4 °C). Homogenates were differentially fractionated according to methods similar to those described by Schmitz et al.[4]. Precipitates from 43,000 × g centrifugation for 20 min twice were used for Mg^{2+}-HCO_3^--ATPase assay. The supernatant from 43,000 × g centrifugation was used for carbonic anhydrase assay.

Mg^{2+}-HCO_3^--ATPase activity was assayed in a manner similar to that described in the previous paper[5]; aliquots of enzyme sample were incubated at 37 °C for 10 min with 1.0 mM $MgCl_2$, 50 mM $NaHCO_3$, 3 mM Na_2ATP (Boehringer, Mannheim) and 50 mM Tris-HCl buffer (pH 9.0). Mg^{2+}-ATPase activity was assayed without HCO_3^-. After incubation, the reaction was stopped by the addition of 20% trichloroacetic acid and liberated inorganic phosphate (Pi) was determined by the method of Allen[6] with a slight modification described by Nakamura[7]. ATPase activity was expressed as μmoles Pi/mg protein/h. Carbonic anhydrase activity was assayed by the modified colorimetric method[8] originated by Wilbur and Anderson[9] using phenol red as indicator. Protein in the enzyme preparation was determined by the method of Lowry et al.[10] with bovine serum albumin as the standard.

Results. The table shows the distribution of Mg^{2+}-HCO_3^--ATPase and carbonic anhydrase activities in the homogenate, microvilli and supernatant fraction of gastrointestinal segments. The highest ATPase activity was found in the duodenal mucosa followed by the jejunal mucosa, while carbonic anhydrase activity was very low in these parts. The



Distribution of Mg^{2+}- and Mg^{2+}-HCO_3^--ATPase and carbonic anhydrase activities in the brush border and supernatant fraction from rat duodenal mucosa. Duodenal segment (15 cm) was excised from the pylorus and cut into 5 segments, 3 cm each in length. The mucosa from 3 rats was combined and used as 1 sample. $NaHCO_3$ concentration used in this case is 100 mM. Each column with vertical lines represents the mean ± SD, n = 3.

Distribution of Mg^{2+}-HCO_3^--ATPase and carbonic anhydrase activities in various organs of rats

Organ	Mg^{2+}-HCO_3^--ATPase (μmoles Pi/mg protein/h)		Carbonic anhydrase (unit/mg protein)	
	Homogenate	Microvilli	Homogenate	Supernatant
Gastric mucosa	10.6± 2.7	19.9± 5.3	91.4±16.4	257.6±71.2
Duodenal mucosa	129.6±10.8	1548.2±80.3	6.1± 0.6	17.5± 2.7
Jejunal mucosa	62.4± 6.9	781.0±53.1	2.9± 0.2	7.5± 1.1
Ileal mucosa	19.5± 3.5	112.5±15.0	1.6± 0.3	3.1± 0.6
Large intestinal mucosa	6.6± 0.7	21.9± 5.1	145.5±23.7	342.6±40.9

Each value represents the mean ± SD, n = 5. Samples from 3 rats were combined and used as 1 sample.

figure shows the more detailed distribution of both enzyme activities in the duodenal mucosa. The highest Mg^{2+}-HCO_3^--ATPase activity was found in the 1st part of the duodenal mucosa (within 3 cm of the pylorus). Carbonic anhydrase activity was also highest in the same area as Mg^{2+}-HCO_3^--ATPase; however, its specific activity was very low.

Discussion. In the present experiment it was demonstrated clearly that the Mg^{2+}-HCO_3^--ATPase activity in the duodenal mucosa, especially in its microvilli, is higher than in the other segments of the gastrointestinal tract investigated. On the other hand, carbonic anhydrase activity was higher in the gastric and large intestinal mucosa, and these distribution patterns were essentially the same as those reported by Carter and Parsons[11]. These phenomena seem to be related to the neutralization of gastric acid. The role of these enzymes may be as follows: CO_2 produced in the duodenal lumen by the neutralization of HCl with $NaHCO_3$ diffuses into the epithelial cells where carbonic anhydrase converts this CO_2 to H_2CO_3 by hydration. H_2CO_3 dissociates to H^+ and HCO_3^-. The former may exchange with Na^+ existing as NaCl in the duodenal lumen and HCO_3^- exchanges with Cl^- through the brush border membrane. Mg^{2+}-HCO_3^--ATPase may mediate this HCO_3^--Cl^- exchange process. Since exchanges of HCO_3^--Cl^- and $H^+ - Na^+$ through the jejunal mucosa have been documented by various investigators[12,13] a similar exchange process may also be expected in the duodenal mucosa. Although the specific activity of carbonic anhydrase in the duodenal mucosa was low, it is interesting to consider that the CO_2 diffusion through the membrane is stimulated by the presence of carbonic anhydrase[14,15].

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Contribution to knowledge of the biosynthesis of cyclosporin A

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Summary. ^3H- and ^{13}C-NMR spectroscopic investigations on the structure of labeled cyclosporin A were performed after feeding of appropriate precursors. The 6 N-methyl groups and the methyl group in position 4 of the ε,ζ-unsaturated amino acid No. 1 (Mebmt) are introduced as intact CH_3-units from methionine. Four head-to-tail acetate units are involved in the biosynthesis of the 8-carbon chain of the olefinic amino acid.

Cyclosporin A (fig. 1), a cyclic peptide with antifungal and immunosuppressive properties, is produced as the major product, together with several minor analogue peptides, in cultures of *Tolypocladium inflatum* Gams. Information on production[2], directed biosynthesis[3], chemistry[4-6] and pharmacology[7,8] has been reported. In feeding experiments with several tritium and carbon-14 labeled precursors [methyl-^3H]methionine proved to be the most suitable compound for the biosynthetic preparation of radiolabeled cyclosporin A for pharmacokinetic and metabolic studies[9]. The analysis of the corresponding ^3H-NMR spectrum (fig. 2), which showed 6 signals for the tritiated 7 N-methyl groups and the methyl group in the γ-position of the olefinic amino acid No. 1, initiated additional more detailed studies on the biosynthesis of cyclosporin A. Accordingly, in a series of experiments the incoorporation of carbon-13 and deuterium from sodium [1-^{13}C]acetate, sodium [2-^{13}C]acetate, [methyl-^{13}C]methionine and [methyl-(^{13}C, $^2H_3$)]methionine was investigated.

Methods. The following nutrient media were used to cultivate the producing organism. Medium 1: malt extract 20 g, yeast extract 4 g, agar 20 g, water to 1 l, pH = 5.7. Medium 2: maltose 50 g, caseinpeptone 10 g, KH_2PO_4 5 g, KCl 2.5 g, water to 1 l, pH = 5.3. A subculture of a strain of *Tolypocladium inflatum* Gams was propagated on medium 1 for 2 weeks at 27 °C and cultivated in medium 2 as earlier described[3]. Filter-sterilized solutions of the precursors were added 2 h after inoculation of the production medium. The cultures were incubated on a rotary shaker for 10 days at 27 °C.

Cyclosporin A was extracted from the broth with 1,2-dichloroethane, purified by gel filtration on sephadex LH 20 with methanol, and separated from minor cyclosporins by repeated HPLC on μ Bondapak Phenyl, 5 μm at 1000 psi with acetonitrile-water 6:4.

Precursors. a) 600 mg/l of sodium [1-^{13}C]acetate at 90 atom % C-13, b) 600 mg/l of sodium [2-^{13}C]acetate at 80 atom % C-13, c) 500 mg/l of [methyl-^{13}C]methionine at 92 atom % C-13, d) 500 mg/l of [methyl-(^{13}C,$^2H_3$)methionine at 92 atom % C-13, 98 atom % ^2H, and e) 75 mg/l of [methyl-^3H]methionine at a sp.act. of 5 Ci/mmole.

Analytical techniques. The incorporation of carbon-13 was determined by ^{13}C-NMR spectroscopy on a Bruker WH-360 spectrometer of solutions of about 250 mg of the corresponding labeled cyclosporin A in 2.5 ml of $CDCl_3$. The relevant signals were assigned by using characteristic