Effect of an Inhibitor of Pepsin Proteolysis in Total Gastric Fistula Dogs

Previous reports from this laboratory have shown that lignosulfonates (sulfonated substituted polymers composed mainly of 3-methoxy-4-hydroxy phenylpropane units[1]) are effective inhibitors of pepsin proteolysis in vitro and in vivo. These materials prevent the development of experimental gastric ulcers in rats[2-4]. The antiulcerogenic activity was ascribed to inhibition of pepsin proteolysis rather than to change in volume or acidity of gastric secretion. In view of these results it seemed desirable to determine the effects of an lignosulfonate in total gastric fistula dogs using coagulated egg albumin as the substrate for pepsin digestion.

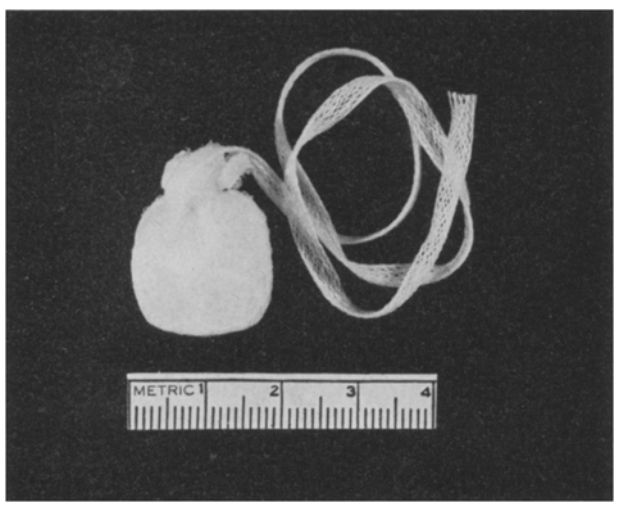

Fig. 1. 4 g of coagulated egg albumin contained in a bag consisting of 3 layers of gauze. The string provided for the positioning of the bag as well as for its removal from the stomach. The bags were weighed on a Sartorius analytical balance prior to and following removal from the stomach.

Materials and methods. Three female mongrel dogs with total gastric fistulas were used. A specially designed cannula provided for placement of the substrate into the stomach and also for easy removal of gastric fluid samples. Following an overnight fast, the animals were given Histalog® (2.0 mg/30 min. s.c.) to elicit gastric secretion. The gastric content samples were removed and treatment administered via a Levin tube (No. 16 French) inserted through the cannula. Following an initial washout period of 1 h, a control gastric sample (3 ml) was obtained by gentle suction. Subsequent 3-ml samples were taken at intervals of 15, 30 and 60 min and all were analyzed for pepsin activity. The pH of the gastric samples was determined by a Radiometer model 28 pH meter. The sodium lignosulfonate (500 mg; designated AHR-2438B) was dissolved in 10 ml water and administered hourly through the Levin tube. 10 ml of water served as placebo.

Two methods were used to determine pepsin proteolysis. In the first, approximately 4 g of coagulated egg albumin was tied into 3 single layers of gauze to form a bag (Figure 1) and secured by a string for inserting through the cannula. The bags were weighed prior to and following removal from the stomach. The substrate was allowed to remain in the stomach for 2 h during control, placebo or treatment periods.

For comparative purposes, pepsin proteolysis was further determined using a modification of the method described by METT[5]. In this procedure, proteolysis is

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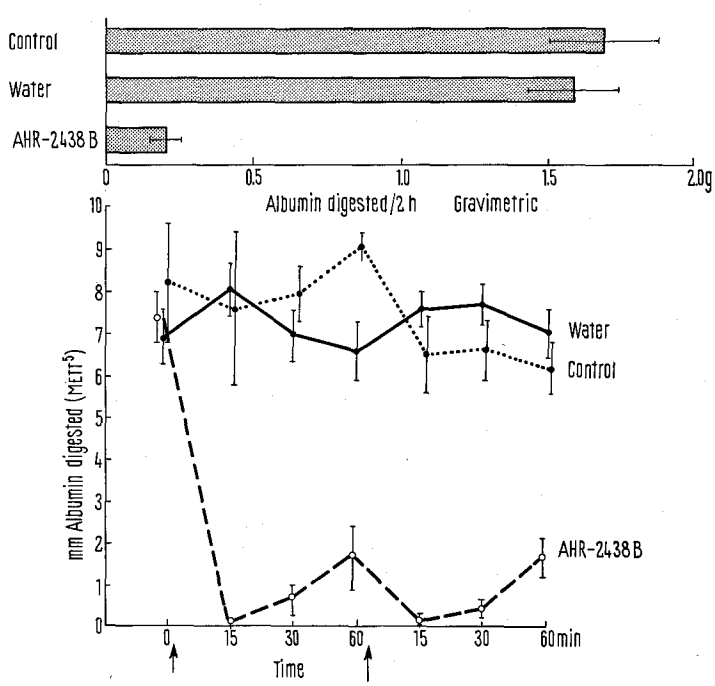

Fig. 2. Effect of AHR-2428 B on pepsin proteolytic activity in 3 gastric fistula dogs. Gastric secretion was elicited by Histalog® (2.0 mg/30 min, s.c.). Placebo (10 ml H_2O) or drug (500 mg in 10 ml H_2O) was administered at time 0 and 60 min. The upper graph shows digestion of egg albumin as determined gravimetrically. Mean values ± S.E. were determined from a total of 16 control, 12 water and 11 AHR-2438-B experiments. The means shown in the lower graph were determined from a range of 7 to 12 values in controls, 11 to 17 in water and 9 to 13 in the AHR-2438 B studies. 3 ml gastric samples were removed by gentle suction at the specified time intervals for pepsin activity determinations.

indicated by the length of coagulated albumin digested in glass tubing of standard diameter under specified conditions[3]. All experiments were randomized in each dog with no animal having more than 2 test days per week.

Results and discussion. Figure 2 shows the effects of AHR-2438B on pepsin proteolysis as determined by the 2 procedures. It is evident that the lignosulfonate markedly inhibited digestion of egg albumin under the conditions described. The maximum effect occurred 15 min postdosing with some inhibition apparent at 60 min. The intragastric administration of 10 ml of water had no effect. The pH values of the gastric samples ranged from 1.0 to 1.3. While the pH of the environment in which pepsin proteolysis occurs is of utmost importance, we have repeatedly demonstrated that such small fluctuations as those observed in these experiments would not be an important factor. Any sample of gastric juice containing bile was discarded.

Results of the present experiments confirm in another animal species that AHR-2438B is an effective inhibitor of pepsin proteolysis and may be useful in the medical treatment of peptic ulcer.

Zusammenfassung. Die Wirkung eines Lignolsulphonates auf die Pepsinproteolyse im Hund mit totaler Magenfistel wurde untersucht und geronnenes Eialbumin als Substrat der Pepsinverdauung verwendet. Es ergibt sich, dass der Pepsin-Inhibitor AHR-2438B – wie früher bei der Ratte nachgewiesen – auch beim Hund die Proteolyse im Magen wirksam hemmt.

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Theophylline-Stimulated Aerobic Glucose Uptake by Rat Thymocytes Exposed to Anoxia[1]

Methyl xanthines have been shown to exert influences on glucose metabolism, but the effects have varied with the tissues and other conditions used. Inhibition of uptake or oxidation of glucose by methyl xanthines has been reported using rat epididymal adipose tissue[2,3], isolated adipose cells[4], polymorphonuclear leucocytes[5], and dog and pig thyroid slices[6]. A biphasic dose-response to theophylline was demonstrated both in sheep thyroid slices[7], and beef thyroid homogenates[8]. This response involved stimulation of $^{14}CO_2$ production from glucose-1-^{14}C at a specific theophylline concentration, and inhibition of the oxidation at higher concentrations.

During studies of aerobic glucose metabolism of isolated rat thymus cells, it was observed that theophylline, at a concentration of 0.5 m*M*, stimulated glucose uptake if the cells had been previously exposed to anoxia. In the present investigation the dose-response curve to theophylline was found to be biphasic, and the effect dependent on both the time of incubation and the glucose concentration.

Materials and methods. Thymocytes were prepared from 150 to 200 g Sprague-Dawley derived male rats (Sasco, Omaha, Nebr.), which were bilaterally adrenalectomized 4 or 5 days before each experiment to remove the source of endogenous steroids which can vary and cause inhibition of the carbohydrate metabolism of these cells[9]. Following decapitation of the rats, thymuses were quickly removed and chilled in Krebs-Ringer bicarbonate (KRB) buffered medium[10], equilibrated with 5% CO_2 in N_2. Cells were released into the medium by gentle teasing, and then filtered through a 200 mesh stainless steel screen, washed twice, and resuspended in KRB buffer to give a 10% (w/v) suspension based on the wet weight of thymus. All measurements are referred to cell number, which was determined by hemocytometry.

Anoxic treatment consisted of a 1 h 37°C incubation of 3×10^8 cells per ml of KRB buffer, gassed with 5% CO_2 in N_2. Aerobic incubations were conducted with 5% CO_2 in O_2 and U-^{14}C-glucose (18 μCi/nmole, New England

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Effect of 0.5 m*M* theophylline on aerobic glucose metabolism of thymocytes following anaerobic preincubation

Theophylline addition	Incubation time (h)	Glucose utilization (μmoles)[a]	Lactate production (μmoles)[a]	$^{14}CO_2$ production (dpm)[a]
0	1	16.52 ± 1.17	15.50 ± 0.15	160,400 ± 4,600
+	1	34.23 ± 1.55[b]	19.43 ± 0.19[b]	165,100 ± 2,00[d]
0	2	28.63 ± 2.86	21.53 ± 1.38	384,400 ± 3,300
+	2	27.50 ± 2.38[d]	26.00 ± 0.54[c]	387,600 ± 5,000[d]
0	3	38.37 ± 1.76	22.07 ± 0.54	564,000 ± 17,900
+	3	34.37 ± 1.87[d]	28.86 ± 0.90[b]	596,500 ± 3,800[d]

[a] Per 10^{10} cells. [b] $p < 0.001$. [c] $p < 0.01$. [d] Not significantly different from the control. Thymocytes (10^8 per ml) in KRB medium were preincubated 1 h at 37°C under 5% CO_2 in N_2 without substrate. Aerobic incubation: 10^8 cells per ml of KRB medium were incubated with 2.78 m*M* U-^{14}C-glucose (49,300 dpm per μmole) at 37°C under 5% CO_2 in O_2. Values are mean of triplicate flasks ± standard deviation.