[Chem. Pharm. Bull. 31(10)3644—3648(1983)]

An Isolated Vascularly Perfused Stomach for Studying Drug Distribution, Metabolism and Action in the Stomach: Acid Secretion in Response to Secretagogues

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(Received February 16, 1983)

Experimental utilization of isolated vascularly perfused rat stomach with retention of the exocrine response to various stimulants was studied. By the inclusion of bovine erythrocytes in the vascular perfusate and by the use of luminal perfusate possessing a suitable buffer capacity, it was found that acid secretion was significantly stimulated by the perfusion of histamine, pilocarpine, cyclic adenosine monophosphate (AMP), dibutyryl cyclic AMP or theophylline. In addition, cimetidine, a histamine H_2-receptor antagonist, inhibited histamine-induced acid secretion in a reversible manner. Although tetragastrin stimulated acid secretion in immature rat or guinea pig stomach, it caused little secretory response in adult rat stomach. On the addition of pepstatin, a pepsin inhibitor, to the vascular perfusate, an acid secretion induced by tetragastrin in adult rat stomach was observed. These findings indicate that some changes in gastrin receptors may develop during the maturation process and that gastrin-induced acid secretion is easily altered by peptic activity in the gastric glands of the perfused stomach. The proposed perfusion system should be useful for studying drug distribution, metabolism and action in the stomach.

Keywords——acid secretion; histamine; cimetidine; gastrin; pepstatin; cyclic AMP; pilocarpine; isolated perfused rat stomach

Since the distribution and metabolism of drugs in certain target tissues are closely related to the pharmacological actions, knowledge of these pharmacokinetic parameters is essential for providing optimal treatment or protection from adverse reactions. By using the organ perfusion technique, we have investigated the tissue distribution and metabolism of drugs in the lung, kidney or pancreas,[1] and have elucidated several possible mechanisms for the disposition of drugs in these tissues. In the case of the stomach, there has been a paucity of information related to pharmacological activity and drug disposition.

The isolated vascularly perfused stomach has been used for studying the gastric endocrine mechanisms,[2-4] but one of the main disadvantages of the perfusion system used was an inability to evoke a measurable response of exocrine secretion to secretagogues. The exocrine response of the vascularly perfused stomach was investigated with the use of a supporting animal, as an oxygenator, in a perfusion circuit[5] or using a stomach pretreated with indomethacin and methysergide.[6] However, these perfusion systems are inappropriate for studying drug distribution and metabolism in the stomach. Van Huis and Kramer[7] have reported that the administration of pilocarpine induces an acid secretion in isolated perfused rat stomach using a perfusion medium containing a fluorocarbon, as a carrier of oxygen, although pentagastrin and histamine failed to stimulate acid secretion in most cases in their experiments.

Therefore, the present study was conducted to develop an experimental isolated vascularly perfused stomach retaining the exocrine response to various secretagogues for the

study of drug distribution, metabolism and action in the stomach.

Methods

Wistar male rats weighing 150—260 g (adult), or 30—50 g (immature), and male guinea pigs (230—260 g) were fasted for about 15 h prior to the experiment, with water *ad libitum*. The stomach vessels were perfused in a nonrecirculating system with Krebs–Ringer bicarbonate buffer (KRBB) supplemented with 25% (v/v) bovine erythrocytes, 4.6% dextran T-70 and 5.8 mM glucose, usually gassed with 95% O_2–5% CO_2. The luminal side of the stomach was perfused with pH 6.6 buffer containing 0.616 mM Na_2HPO_4, 0.092 mM citric acid and 154 mM NaCl.[8]

Animals were anesthetized with pentobarbital (40 mg/kg, intraperitoneally), after which the trachea was exposed and the vagus was severed. The abdomen was opened by midline incision, the superior mesenteric and splenic arteries were ligated, and the abdominal aorta was cannulated with polyethylene tubing (o.d.: 1.2 mm) after the spleen had been removed. The stomach was cannulated with esophageal tubing (o.d.: 2 mm) which was secured by a ligature around the cardia, and another cannulation was performed at the pylorus. After flushing of the gastric lumen with physiological saline, it was perfused with pH 6.6 buffer having the components described previously[8] at the rate of 1.0 ml/min (0.5 ml/min for immature rat stomach). The pyloric cannula was connected to a glass electrode through a short piece of tubing (o.d.: 2 mm). A report[8] appeared previously on the titration curve of this buffered perfusate. An approximately linear relationship between the amount of HCl added and the pH changes in the range from 3.8 to 6.6 was observed. The time integral of pH deflection was therefore expected to be proportional to the acid output. Thus, in this study, stimulated acid secretion was expressed as pH deflection using this buffer and acid output determined by titration with 0.01 N NaOH to pH 6.6 using a pH-stat (Toa Electronics HSM-10A). When a plateau of acid secretion was achieved, heparin (500 U/kg) was injected through the cannula of the abdominal aorta. The right and left renal arteries, pancreaticoduodenal artery, hepatic artery and portal vein were ligated. As soon as the stomach vessels were perfused at the rate of 0.7 ml/min (0.2 ml/min for immature rat stomach) *via* a peristaltic pump, the aorta was ligated just above the origin of the coeliac artery, and the inferior portal vein was cannulated (o.d.: 1.5 mm). The venous effluents were collected through this portal cannula. Both the perfusates and the stomach were kept at 37 °C throughout the experiments.

Drugs used in this paper were as follows: histamine diphosphate and pilocarpine hydrochloride (Nakarai Chemicals), theophylline (Wako Pure Chemical), cyclic adenosine monophosphate (AMP) and dibutyryl cyclic AMP (Sigma), cimetidine (Smith Kline & Fujisawa), tetragastrin (San-a Pharmaceutical), pepstatin (Protein Research Foundation). All other chemicals used were of analytical grade and commercially available.

Results and Discussion

It is known that parietal cells are susceptible to hypoxic damage,[7] and thus we examined the effect of oxygen on the acid secretion of isolated perfused rat stomach in order to establish the state of the vascular perfusate. For this study, O_2-saturated dextran-KRBB solution, O_2-

Table I. Stimulation of Acid Secretion by Various Stimulants in Isolated Perfused Rat Stomach

Stimulant	Concentration	Acid output (μeq/30 min)
Histamine	24 μM	0.95±0.08[a]
Histamine	33 μM	2.90±0.28
Histamine	65 μM	4.44±0.74
Histamine	130 μM	4.29±0.53
Pilocarpine	2.5 μM	0.38±0.21
Pilocarpine	5.0 μM	0.74±0.22
Cyclic AMP	1.0 mM	0.30±0.07
Cyclic AMP	2.0 mM	0.67±0.09
Cyclic AMP	4.0 mM	2.53±0.84
Dibutyryl cAMP	0.1 mM	1.00±0.58
Dibutyryl cAMP	0.2 mM	4.77±0.76
Dibutyryl cAMP	0.4 mM	4.63±1.47
Theophylline	5.6 mM	0.78±0.30

Perfusion time of stimulant: 10 min.
a) Mean±S.E. of 3—4 experiments.

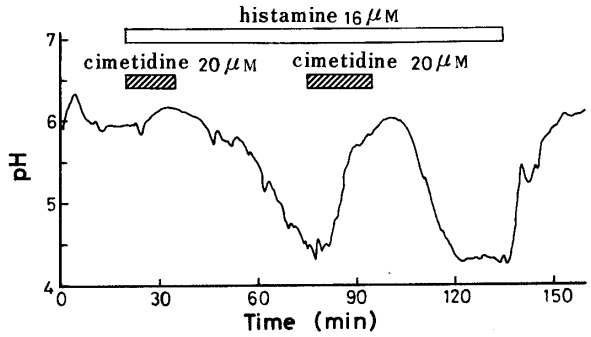

Fig. 1. Effect of Cimetidine on Acid Secretion Induced by Histamine in Isolated Perfused Rat Stomach

A representative example of three experiments is shown.

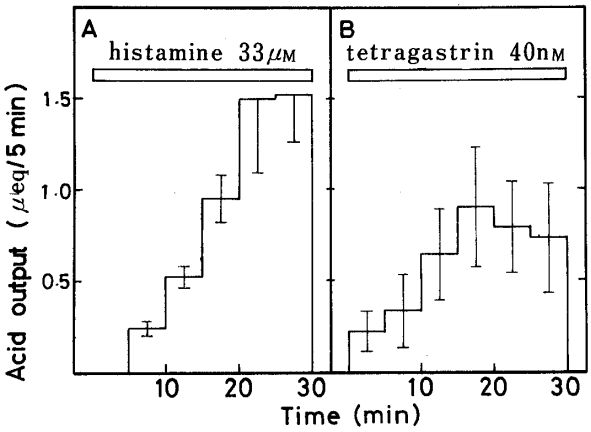

Fig. 3. Effects of Histamine and Tetragastrin on Acid Secretion in Isolated Perfused Stomach of Adult Rat

Histamine (A) or tetragastrin (B) was perfused for 30 min in the presence of 1 μM pepstatin. The results represent the mean ± S.E. of three (histamine) or seven (tetragastrin) experiments after subtracting basal secretion.

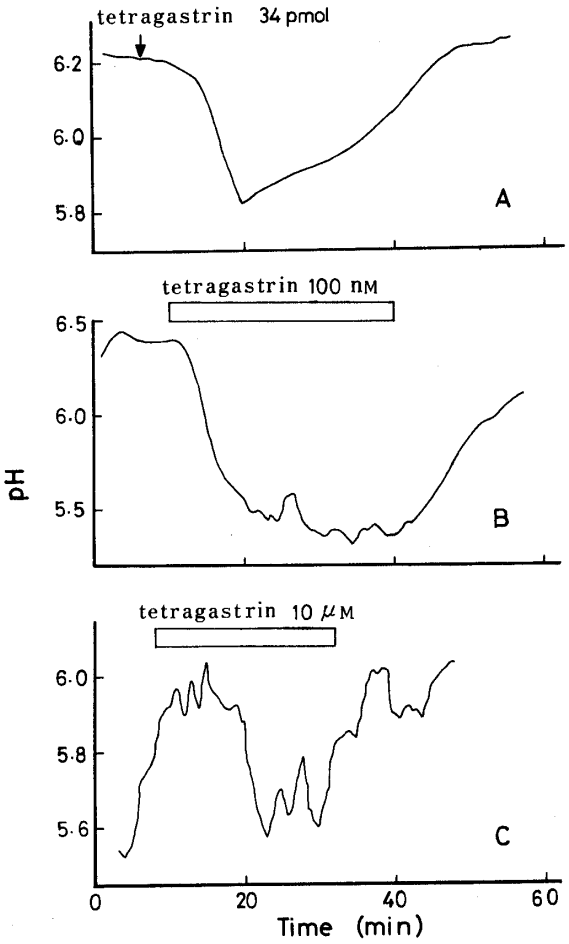

Fig. 2. Effect of Tetragastrin on Acid Secretion in Isolated Stomach

A) Immature rat (32 g of body weight) stomach was perfused without pepstatin. B) Adult rat (170 g) stomach was perfused with 1 μM pepstatin. C) Guinea pig (260 g) stomach was perfused without pepstatin. These data are representative of 3—7 experiments.

saturated dextran-KRBB suspension containing 25% bovine erythrocytes, and N_2-saturated dextran-KRBB-erythrocytes suspension were compared. Histamine-induced acid secretion was observed only in O_2-saturated KRBB containing 25% bovine erythrocytes. This result indicated that acid secretion required a considerable amount of oxygen, and that erythrocytes were necessary as an oxygen carrier.

To ascertain the exocrine function of the designed perfused stomach, the effect of various stimulants on the acid secretion was examined. When histamine, pilocarpine, cyclic AMP, dibutyryl cyclic AMP or theophylline was used as a secretory stimulant, a marked increase in acid secretion was obtained (Table I). Dose-dependent acid secretion was demonstrated in response to histamine, cyclic AMP and dibutyryl cyclic AMP. However, the molar concentration of cyclic AMP required for the increase in acid secretion was many times greater than that of dibutyryl cyclic AMP. This result is consistent with the reported slow penetration of cyclic AMP into cells.[9]

The effect of a histamine H_2-receptor antagonist, cimetidine, on histamine-induced acid secretion was also investigated. Figure 1 shows that cimetidine inhibited the acid secretion induced by histamine in a reversible manner.

Despite the good response of acid secretion to histamine, pilocarpine, cyclic AMP and theophylline, the isolated stomach of adult rat hardly responded to tetragastrin, an active peptide fragment of gastrin. However, a response was obtained in immature rat stomach by the administration of tetragastrin, as shown in Fig. 2A. The defect of response to tetragastrin in the adult rat stomach indicated that some physiological changes in the receptor for gastrin or in peptic activity in the gastric glands may occur during the development. Furihata *et al.*[10] reported that the peptic activity of pepsinogen in the gastric mucosa remained constant at approximately 20% of that of an adult rat up to 17 d, then increased sharply from the 18th d, reaching the adult level on the 30th d. In order to clarify the relation between the acid secretion and peptic activity in the gastric glands, the effect of pepstatin, a typical pepsin inhibitor,[11] on the acid secretion was investigated. Figure 2B illustrates the acid output stimulated by tetragastrin in adult rat stomach perfused with a medium containing pepstatin (1 μM). It is possible that acid secretion induced by tetragastrin would be sensitive to pepsin or pepsin-like acid proteases. To confirm this phenomenon, we studied the response to tetragastrin in the isolated stomach of guinea pig, which has low peptic activity in the gastric mucosa as compared to that of rat (unpublished data). As shown in Fig. 2C, it was found that tetragastrin stimulated acid secretion in the perfused guinea pig stomach even in the absence of pepstatin.

The time course of acid output induced by histamine or tetragastrin is presented in Fig. 3. Histamine and tetragastrin induced a rapid increase in acid output, although marked acid secretion was not observed within the initial 5 min of histamine perfusion. The more rapid onset of acid secretion associated with tetragastrin is consonant with the observation of James, who found pentagastrin to be more rapidly effective than histamine in *in vivo* experiments.[12]

It has been difficult to obtain a response to gastrin in the *in vitro* system including vascularly perfused stomach,[5,7] isolated stomach preparation from adult mammals,[13] isolated gastric glands,[14] and isolated parietal cells.[15] The present study suggests that the response of acid secretion to gastrin is altered by maturation, species difference, and peptic activity in gastric glands. In this new preparation, acid secretion could be observed for 2 h without the development of edema. Kowalewski and Sharf[5] reported that a bubble oxygenator could not be used for prolonged perfusion, and that if this is needed a supporting animal should be used in the perfusion circuit. However, in our experiment with a nonrecirculating system, the evidence indicated that a supporting animal was not essential.

The data gathered in the present study suggest that the new isolated perfused stomach is an appropriate system for the investigation of drug distribution and metabolism in the stomach, as well as for studies on pharmacological effect, since an almost normal physiological function was retained.

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