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Regulatory mechanism of duodenal bicarbonate secretion Roles of endogenous prostaglandins and nitric oxide

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ABSTRACT

The secretion of HCO_3^- in the duodenum is increased by exogenous prostaglandin (PG) E_2 and mucosal acidification, the latter being accompanied by a rise in mucosal PGE_2 content and nitric oxide (NO) release. The stimulatory effect of PGE_2 is mediated intracellularly by both Ca^{2+} and 3′,5′-adenosine cyclic adenosine monophosphate (cAMP), and this action is inhibited by EP3 and EP4 antagonists. The secretion is also increased by NOR3 (NO donor), and this response is mimicked by dibutyryl 3′,5′-cyclic guanosine monophosphate (dbcGMP) and attenuated by indomethacin. Mucosal acidification stimulates HCO_3^- secretion with concomitant increases in mucosal PGE_2 production and NO release. The effects on HCO_3^- secretion and PGE_2 production are inhibited by indomethacin [nonselective cyclooxygenase (COX) inhibitor] and SC-560 (selective COX-1 inhibitor) but not rofecoxib (selective COX-2 inhibitor). N^G-nitro-L-arginine methyl ester [L-NAME: nonselective NO synthase (NOS) inhibitor], but not aminoguanidine [selective inducible NOS inhibitor], attenuates the acid-induced HCO_3^- secretion and NO release in an L-arginine-sensitive manner. In addition, the response to PGE_2 is potentiated by vinpocetine [phosphodiesterase (PDE) 1 inhibitor] and cilostamide (PDE3 inhibitor), while the response to NOR3 is increased by vinpocetine. We conclude that endogenous PGs and NO are both involved in the local regulation of acid-induced duodenal HCO_3^- secretion; COX-1 and constitutive NOS are key enzymes responsible for the production of PGs and NO, respectively; NO stimulates HCO_3^- secretion by increasing PG production; PGE_2 stimulates HCO_3^- secretion via activation of EP3/EP4 receptors; and both PDE1 and PDE3 are involved in the regulation of duodenal HCO_3^- secretion.

Contents

Abbreviations: AC, adenylate cyclase; cNOS, constitutive nitric oxide synthase; cAMP, 3′,5′-cyclic adenosine monophosphate; cGMP, 3′,5′-cyclic guanosine monophosphate; COX, cyclooxygenase; CFTR, cystic fibrosis transmembrane conductance regulator; dbcAMP, dibutyryl 3′,5′-cyclic adenosine monophosphate; dbcGMP, dibutyryl 3′,5′-cyclic guanosine monophosphate; GC, guanylate cyclase; iNOS, inducible nitric oxide synthase; IBMX, isobutylmethylxanthine; NO, nitric oxide; L-NAME, N^G-nitro-L-arginine methyl ester; PI, phosphatidyl inositol; PDE, phosphodiesterase; PG, prostaglandin; PGI_2, prostacyclin; TRPV1, transient receptor potential vanilloid type 1.

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1. Introduction

Duodenal mucosal HCO_3^- secretion is a key process that aids in preventing acid-peptic injury (Flemstrom & Garner, 1982; Takeuchi et al., 1986a, 2010; Takeuchi & Okabe, 1995). Hydrogen ions within the lumen are removed from the proximal duodenum through the neutralization of acid by HCO_3^- that originates from the duodenal epithelium. Mucus adherent to the luminal surface of the mucosa provides a zone of low turbulence (unstirred layer), allowing the development of a gradient for HCO_3^- from the luminal side (Takeuchi et al., 1983). Small amounts of HCO_3^- protect the mucosa against large amounts of acid by neutralizing H^+ ions that diffuse back into the mucus layer, and hence the duodenal HCO_3^- secretion is thought to play an important role in the pathogenesis of experimental and clinical duodenal ulcers (Briden et al., 1985; Takeuchi et al., 1986a, 1986b). Notably, the ability of the mucosa to respond to acid seems very important in the maintenance of the surface pH gradient and in the protection of the mucosa (Heylings et al., 1984). The mechanisms that govern mucosal HCO_3^- secretion involve neuro-humoral factors and luminal acid (Flemstrom & Garner, 1982; Takeuchi & Okabe, 1995; Takeuchi et al., 1990, 1991; Hogan et al., 1993), yet it is thought that endogenous prostaglandins (PGs) and nitric oxide (NO) are particularly important in the local control of this secretion.

A variety of substances including PGE_2, vasoactive intestinal peptide, theophyline, forskolin and pituitary adenylate cyclase activating peptide are shown to stimulate duodenal HCO_3^- secretion both *in vivo* and *in vitro* (Flemstrom, 1980; Simson et al., 1981; Hogan et al., 1993; Takeuchi et al., 1997a, 1997b). These agents increase intracellular levels of adenosine-3′,5′-cyclic monophosphate (cAMP) by stimulating adenylate cyclase (AC) or inhibiting phosphodiesterase (PDE), suggesting cAMP to be a mediator of HCO_3^- secretion in the duodenum. Furthermore, studies demonstrated that guanylin, an endogenous activator of guanylate cyclase (GC), increases HCO_3^- secretion in the rat duodenum via guanosine-3′,5′-cyclic monophosphate (cGMP) and that NOR3, a NO donor [(±)-(E)-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamine], stimulates duodenal HCO_3^- secretion in several species, partly mediated by endogenous PGs (Guba et al., 1996; Furukawa et al., 1999; Sugamoto et al., 2001). It has also been reported that NO donors stimulate PG production in several organs and several types of cells including the gastrointestinal epithelial cells (Salvemini et al., 1994; Wilson et al., 1996; Uno et al., 1997). In addition, since HCO_3^- secretion in the duodenum is intracellularly mediated by both cAMP and cGMP (Simson et al., 1981; Guba et al., 1996; Takeuchi et al., 1997a; Furukawa et al., 1999), it is possible that PDE affects the response by altering the levels of cyclic nucleotides.

In this article, we reviewed our publications on the roles of endogenous PGs and NO in the regulatory mechanism of duodenal HCO_3^- secretion, including EP receptor subtypes, cyclooxygenase (COX) and NO synthase (NOS) isozymes as well as the interaction of PGs and NO (Takeuchi et al., 1997b, 1999a, 2002, 2003; Sugamoto et al., 2001; Aoi et al., 2004; Aihara et al., 2007). In addition, we also introduced which isozyme(s) of PDE is involved in the local regulation of duodenal HCO_3^- secretion (Hayashi et al., 2007; Kita et al., 2008).

2. Experimental system for measuring duodenal HCO_3^- secretion

The data presented in this article were obtained in rat or mouse duodenums *in vivo* and amphibian duodenums *in vitro*. To aid in understanding our data, we briefly described our experimental systems for measuring duodenal HCO_3^- secretion.

2.1. In vivo system

Under urethane anesthesia (1.25 g/kg, i.p.), a duodenal loop (1.7 cm in rats or 8 mm in mice) was made between the pyloric ring and the area just above the outlet of the common bile duct in order to exclude the influences of bile and pancreatic juice (Takeuchi et al., 1986a) [Fig. 1A]. Then, the loop was perfused with saline, and HCO_3^- secretion was measured at pH 7.0 by using the pH-stat system and by adding 2 or 10 mM HCl to the reservoir.

2.2. In vitro system

Bullfrogs were pithed, and the duodenal mucosa was stripped from the muscle layer by blunt dissection. The tissues were mounted

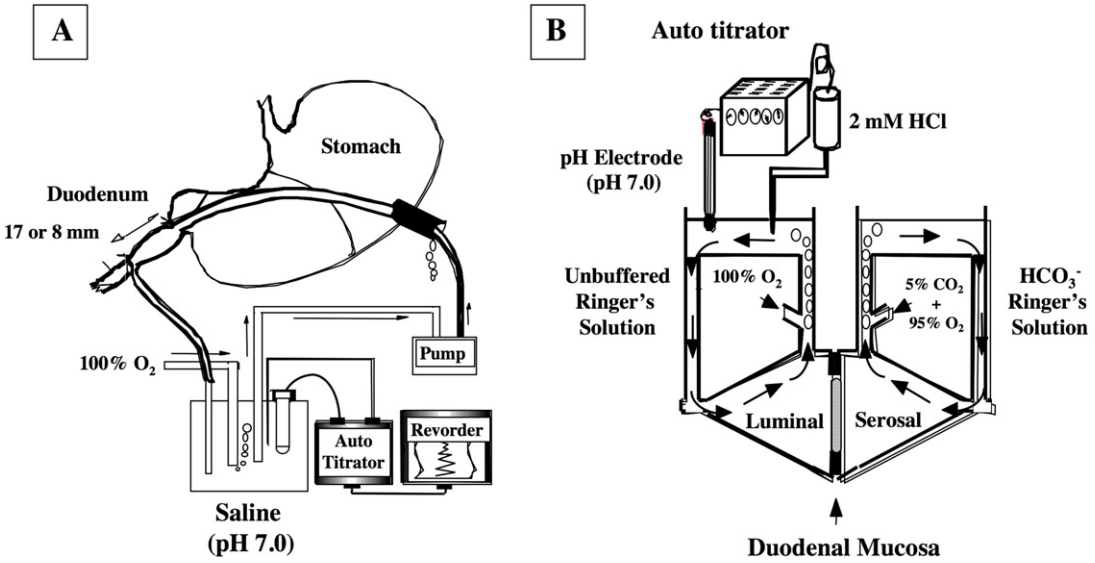

Fig. 1. The perfusion system and order of connections for measuring HCO_3^- secretion in the anesthetized rat or mouse duodenum (**A**) and in the isolated bullfrog duodenum (**B**). In Fig. A, a duodenal loop was made between the pylorus and the area just above the outlet of the common bile duct, and perfused with saline at a flow rate of 1 ml/min. In Fig. B, the duodenum was isolated and mounted between two halves of a lucite chamber (the exposed area was 0.6 cm^2). The tissue was bathed in 120 mM NaCl gassed with 100% O_2 on the mucosal side and HCO_3^- Ringer's solution gassed with 95%O_2–5% CO_2 on the serosal side, and these solutions were continuously circulated by a gas-lift system. To determine the response, an automatic titrator was introduced into the perfusion system, and HCO_3^- secretion was measured at pH 7.0 using the pH-stat method and by adding 10 or 2 mM HCl. Data adopted after modification from Refs.Takeuchi et al. (1997b) and (2010).