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Acid Secretion and Nitric Oxide Synthase Activity in Gastric Glands Following Hypoxia/Reoxygenation and Acidosis

Hyeyoung Kim and Kyung Hwan Kim

Department of Pharmacology, Yonsei University College of Medicine, Seoul 120-752, Korea

ABSTRACT

Acid secretion and NO synthase activity were determined in isolated gastric glands following hypoxia/reoxygenation and acidosis to investigate the involvement of NO in acid secretion. Isolated gastric glands were exposed to hypoxia (30 min)/reoxygenation (1 h) and/or to acidosis (pH 6.0 and 4.0). Acid secretion was measured by the ratio of [^{14}C]-aminopyrine accumulation between intra- and extraglands. NO synthase activity was determined by percent conversion to [^{14}C]-L-citrulline from [^{14}C]-L-arginine, a precursor of NO. The results indicate that dibutyryl cAMP stimulated acid secretion dose-dependently but had no effect on NO synthase activity in basal gastric glands. Hypoxia/reoxygenation significantly suppressed acid secretion both in unstimulated and stimulated gastric glands, which was exaggerated by acidosis. Constitutive NO synthase activity, not responded to dibutyryl cAMP, was also inhibited by hypoxia/reoxygenation and acidosis. In conclusion, pathologic state of gastric mucosa such as hypoxia/reoxygenation and acidosis suppresses both acid secretion and NO release but the role of NO in acid secretion stimulated by dibutyryl cAMP in basal gastric glands is not significant.

Key Words: Acid secretion, Nitric oxide synthase, Hypoxia/reoxygenation, Acidosis, Gastric gland

INTRODUCTION

The ischemia or reduced gastric perfusion is known to be associated with mucosal injury in several different clinical settings including trauma (Lucas *et al.*, 1971), major surgery (Lucas *et al.*, 1971) and nonsteroidal antiinflammatory drug administration (Kitahora and Guth, 1987). Increasing evidence suggests that majority of injury occurs during reperfusion (Perry *et al.*, 1986; Perry and Wadhwa, 1988; Andrewes *et al.*, 1992) and this may be contributed by reactive oxygen metabolites initially generated at the level of the vasculature (Tsao *et al.*, 1990). Prolonged ischemia itself results in injury due to oxygen deprivation. However, shorter periods of ischemia initiate the production of toxic reactive oxygen metabolites and causes cellular changes when the tissue is reoxygenated (McCord, 1985). Sources of reactive oxygen metabolites in reperfusing tissues include the xanthine oxidase system, which is modified during ischemia such that produces O_2^- and H_2O_2 during reperfusion (Parks and Granger, 1986), and activated neutrophils, which infiltrate the tissues and bind to the microvascular endothelium (Grisham *et al.*, 1986; Hernandez *et al.*, 1987).

Because hypoxia has been shown to produce H^+ within the cytoplasm as a result of anaerobiosis in a variety of tissues (Hochachka and Mommsen, 1983), it is reasonable to assume that acidosis exaggerates hypoxic cell injury by accentuationg intracellular acidosis. Acidosis activates the release

of chemical mediators such as histamine (Rangachari, 1975), leukotriene (Wallace *et al.*, 1990), platelet-activating factor (Kubes *et al.*, 1990), which might accentuate cell injury during hypoxia or reperfusion.

Nitric oxide (NO) is emerging as an important endogenous vasodilator in the gastric vasculature (Whittle *et al.*, 1990). It appears to play a protective role in the gastric mucosa since it is involved in the hyperemic response to damaging agents (Lippe and Holzer, 1992). Nitric oxide may modulate gastric mucosal integrity by interacting with other protective mediators such as sensory neuropeptides and endogenous prostaglandins (Pique *et al.*, 1989; Whittle *et al.*, 1990; Tepperman and Whittle, 1992) and secretion of mucus or bicarbonate by a cyclic GMP-dependent process (Flemstrom, 1987). These act as a first line of mucosal defense against luminal aggressors in the stomach. The role of nitric oxide in ischemia/reperfusion has not been clarified. The possible protective mechanisms of nitric oxide on the gastric mucosa during ischemia-reperfusion injury are: (1) vasodilation improving perfusion of the tissues, (2) inhibition of platelet (Radomski *et al.*, 1987) or polymorphonuclear leukocyte adherence to the endothelium (Kubes *et al.*, 1991), and (3) scavenging of the superoxide radical (Gryglewski *et al.*, 1986).

Therefore, the present study was designed to investigate the involvement of NO in acid secretion during ischemia/reperfusion injury and acidosis in isolated gastric glands.

MATERIALS AND METHODS

Chemicals

[^{14}C]-L-Arginine monohydrochloride (300 mCi/mmol) and [dimethylamine -^{14}C]-aminopyrine (109 mCi/mmol), Amersham International Place (Buckinghamshire, England); Dowex AG 50WX-8, Bio-Rad Laboratories (Richmond, CA); collagenase (Type I), rabbit albumin, β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt (NADPH), N^6, 2'-O-dibutyryl adenosine 3',5'-cyclic monophosphoric acid, sodium salt and all other chemicals, Sigma Chemical Co. (St. Louis, MO).

Solutions

Collagenase enzyme solution was composed of NaCl 130. mM, $NaHCO_3$ 12.0 mM, NaH_2PO_4 3.0 mM, K_2HPO_4 3.0 mM, $MgSO_4$ 2.0 mM, $CaCl_2$ 1.0 mM, Phenol red 10 mg/l, pH 7.4. Before use 1 mg/ml collagenase, 1 mg/ml rabbit albumin and 2 mg/ml glucose were added. The incubation medium consisted of NaCl 132. 4 mM, KCl 5.4 mM, Na_2HPO_4 5.0 mM, NaH_2PO_4 1.0 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.0 mM, phenol red 10 mg/l, pH 7.4. Before use 2 mg/ml rabbit albumin and 2 mg/ml glucose were added.

Animals

Male New Zealand white rabbits weighing 1.5~2.5 kg were used. The rabbits were not starved. Anesthesia was induced with 30 mg/kg secobarbital sodium intravenously, the abdomen was opened and the aorta was cannulated in a retrograde directon. 5 ml of heparin, 250 U/ml, were injected with force through the cannula. After one minute the rabbit was bled through the cannula and a ligature was placed around the mesenteric vessels. The chest was quickly opened and the thoracic aorta clamped. A warm 37℃, oxygenated PBS was then pumped into the aorta, whereupon the portal vein was opened to allow a free outflow of the perfusate. By this procedure most of the solution was forced through the gastric blood vessels. When the stomach appeared totally exsanguinated after perfusion of some 500 ml PBS, it was rapidly removed, cut open along the lesser curvature and emptied.

Separation of gastric glands

Gastric gland was separated by the method of Berglindh and Obrink (1976). Briefly, the cardial and antral regions were discarded. The corpus was rinsed several times in PBS and finally blotted with a filter paper, whereby the remaining gastric content as well as some surface epithelial cells were removed. By blunt dissection the mucosa could easily be separated from the muscular and submucosal layers. It was then minced into small pieces with a pair of scissors. The pieces were washed twice in warm oxygenated PBS and transferred to a 200 ml flask containing 50 ml collagenase-enzyme solution. The flask was gassed

with 100% oxygen, sealed and put into a 37℃ water bath. During the following incubation the content was gently stirred with a magnet. The whole procedure from the removal of the stomach until the start of the incubation took less than 5 min. After 90 min, when a large number of gastric glands as well as some cells had been separated, the incubation was terminated and the rest of the procedure was continued at room temperature. The cloudy suspension was filtered through a nylon mesh into 15 ml test tubes with conical bottoms. The glands were washed free from isolated cells and collagenase and the washing was performed with incubation medium three times in about 15 min. The yield of gastric glands from the perfused mucosa of each corpus was approximately 750 mg wet weight. Intact morphology was examined under light microscope and viability (>90%) was cheked by trypan blue exclusion assay.

Experimental protocol: hypoxia/reoxygenation and acidosis

Glands were resuspended in incubation medium containing 10 mM Hepes, pH adjusted to 7.4, 6.0, and 4.0, to 20~25 mg/ml and hypoxia was induced by 100% N_2 for 30 min at 37℃. Control glands were continuously oxygenated. Gastric suspension prepared in each pH and received hypoxia or normoxia for 30 min, further incubated for 1 h at 37℃ under oxygen supply following treatment of either [^{14}C]-L-arginine and Ca/NADPH for nitric oxide synthase assay or dibutyryl cAMP and [^{14}C]-aminopyrine for acid secretion assay. Finally whole procedure induced hypoxia (30 min)/reoxygenation (1 h) with or without acidosis. Control glands, in pH 7.4, 6.0 and 4.0, were continuously oxygenated for 90 min. In present study, normoxic control is considered as continuously oxygenated gastric glands.

Determination of [^{14}C]-aminopyrine accumulation

The ability of gastric glands to secrete acid was used as an indicator of normal glandular function (Berglindh *et al.*, 1976). After either hypoxia or normoxia for 30 min at 37℃, glandular suspension was treated with [^{14}C]-aminopyrine (0.2 μCi/ml) with (stimulated) or without 1 mM dibutyryl cAMP (unstimulated). Incubation was continued for 1 h at 37℃ under continuous oxygenation. The incubation was ended by centrifuging the glands in an Eppendorf microfuge for 2 min and supernatant was placed in a scintillation vial. The pellet was solubilized with 1N NaOH and radioactivity was counted. Aminopyrine accumulation was determined as the ratio of intra-to extracellular aminopyrine.

Assay of NO synthase

NO synthase was measured by determining the production of [^{14}C]-L-citrulline, which is a by-product of the enzyme reaction from [^{14}C-]-L-arginine. After 30 min of hypoxia or normoxia, gastric glandular suspension (20~25 mg gastric glands/ml) was incubated with 0.45 mM calcium, 1 mM NADPH and [^{14}C]-L-arginine (20 nCi/ml)at 37℃ for 1 h under oxygen supply. The reaction was stopped by addition of 1.5 ml of ice-cold Hepes/EDTA buffer (20 mM/2 mM, pH 6.0), sonicated (10 sec, three times) and centrifuged (10,000 xg for 15 min at 4℃). The supernatant applied to a 1-ml Dowex 50 cation exchange column. [^{14}C]-L-citrulline in the effluent and a subsequent 1 ml water wash was quantified by liquid scintillation spectrometry (Kim *et al.*, 1992). The sole radioactive component was verified as [^{14}C]-L-citrulline by thin layer chromatography with solvent system of chloroform: methanol: ammonium hydroxide: water (0.5: 4.5: 2.0: 1.0) (data not shown). Percent conversion, as an index of NO synthase activity was calculated as dpm of [^{14}C]-L-citrulline/dpm of [^{14}C]-L-arginine×100.

Measurement of cell viability

There is no universally accepted definition of cell death, and common methods of estimation of cell death include morphological analysis, measurement of the release of cytoplasmic enzymes, or uptake of fluorescent compounds (Cook and Mitchell, 1989). Since release of lactic dehydrogenase (LDH) reflects permeability of the cytoplasmic membrane, LDH release in glandular suspension in each pH, received either hypoxia/reoxygenation or normoxia was measured by the method of Babson and Phillips (1965). In additional experiment, various proportions of sonicated glands and freshly prepared glands were mixed and LDH release was measured to detrermine that LDH is an useful index of glandular cell viability in this study.

Statistics

All values represent mean±S.E.M. from 5 experiments. Differences among groups were determined by neo-way ANOVA with Newman-Keuls test (Zar, 1984). Values were considered significantly different if $P<0.05$.

RESULTS

Dose response curve of dibutyryl cAMP for acid secretion of gastric glands

For response study of gastric glands to dibutyryl cAMP, isolated gastric glands were suspended in incubation medium containing 10 mM Hepes, pH 7.4 to 20~25 mg/ml and continuously oxygenated for 30 min at 37°C. Various concentration of dibutyryl cAMP (final concentration 10^{-4} M-10^{-3} M) and [^{14}C]-aminopyrine (0.2 μCi/ml) were treated to glandular suspension to stimulate acid secretion. After further 1 h incubation at 37°C under oxygen supply, dibutyryl cAMP stimulated [^{14}C]-aminopyrine uptake dose-dependently (Fig. 1). With treatment of dibutyryl cAMP, the ratio of intra-to extracellular aminopyrine accumulation was increased to 120±4%, 145±5%, 220±15%, 240±15% and 250±20% of nontreated control at 10^{-4} M, 2.5×10^{-4} M, 5×10^{-4} M, 7.5×10^{-4} M and 10^{-3} M dibutyryl cAMP, respectively. Thus 10^{-4} M dibutyryl cAMP was used for next series of experiments to stimulate acid secretion.

Effects of hypoxia/reoxygenation and acidosis on acid secretion of unstimulated and stimulated gastric glands

In continuous oxygenated condition, considered as normoxia in present study, [^{14}C]-aminopyrine accumulation ratio of unstimulated gastric glands incubated at pH 7.4 was 2.5±0.09% and increased to 6.25±0.1% (250% of unstimulated gastric glands) by 10^{-3} M of dibutyryl cAMP(Fig. 2). This

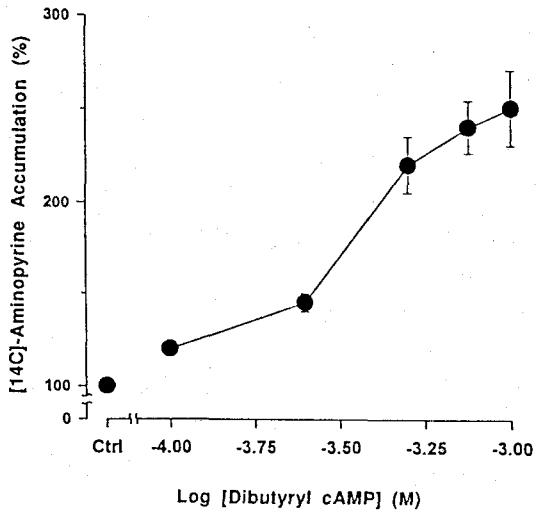

Fig. 1. Dose response curve of dibutyryl cAMP for acid secretion of gastric glands. Dibutyryl cAMP (final concentration 10^{-4} M -10^{-3}M) and [^{14}C]-aminopyrine (0.2 μCi/ml) were treated to glandular suspension to stimulate acid secretion. Acid secretion was determined by the ratio of [^{14}C]-aminopyrine accumulation between intra- and extraglands. Each point represents mean±S.E.M.(n=5) Ctrl, control.

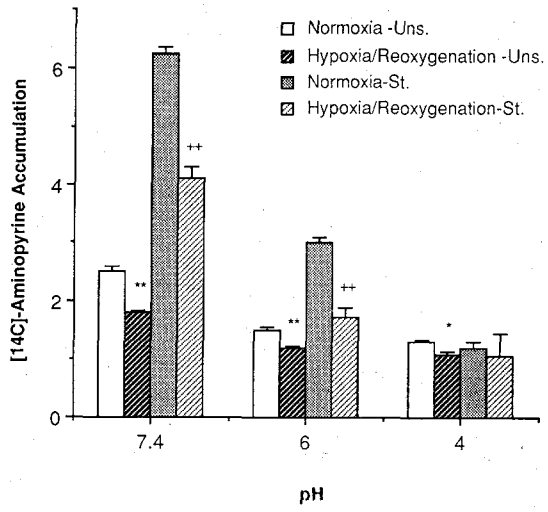

Fig. 2. Effects of hypoxia/reoxygenation and acidosis on acid secretion of unstimulated and stimulated gastric glands. Each bar represents mean±S.E.M. (n=5). *P<0.05, **P<0.01; compared with normoxic unstimulated gastric glands. $^{++}$P<0.01; compared with normoxic stimulated gastric glands. Uns., unstimulated gastric glands; St., stimulated gastric glands.

stimulation of acid secretion was inhibited by hypoxia (30 min)/reoxygenation (1 h), but dibutyryl cAMP signficantly increased [^{14}C]-aminopyrine uptake in gastric glands incubated at pH 7.4 even in the state of hypoxia/reoxygenation; [^{14}C]-aminopyrine accumulation ratio of hypoxic / reoxygenated gastric glands were 1.8±0.04%(unstimulated) and 4.1±0.2% (stimulated gastric glands). At pH 6.0, dibutyryl cAMP stimulated acid secretion to 200% of unstimulated normoxic gastric glands, which also inhibited by hypoxia/reoxygenation. The ratio of [^{14}C]-aminopyrine accumulation of unstimulated normoxic, stimulated normoxic, unstimulated hypoxic/reoxygenated and stimulated hypoxic/reoxygenated gastric glands were 1.5±0.05%, 30.±0.01%, 1.2±0.04% and 1.74±0.17%, respectively. Between pH 7.4 and 6.0, both stimulatory effect of dibutyryl cAMP and inhibition by hypoxia/reoxygenation on acid secretion showed similar pattern even though absolute values were significantly different. However, glandular function was significantly reduced by acidosis (pH 4.0), which was proved by little responses by dibutyryl cAMP and hypoxia/reoxygenation. [^{14}C]-Aminopyrine accumulation ratio of all glands were similar as 1.3±0.035% (unstimulated normoxic), 1.1±0.04% (stimulated normoxic), 1.2±0.12% (stimulated hypoxic/reoxygenated) and 1.05±0.4% (stimulated hypoxic/reoxygenated gastric glands).

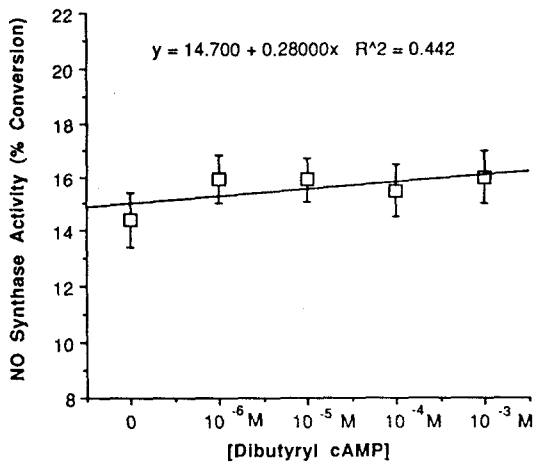

Fig. 3. Effect of dibutyryl cAMP on NO synthase activity of gastric glands. Gastric glands were incubated with [^{14}C]-L-arginine and dibutyryl cAMP (10^{-6} M−10^{-3} M) and NO synthase activty was calculated as percent conversion to [^{14}C]-L-citrulline from [^{14}C]-L-arginine, a precursor of NO. Each point represents mean ± S.E.M. (n=5). No significant difference was found between treatment of dibutyryl cAMP and NO synthase activity.

Effect of dibutyryl cAMP on NO synthase activity of gastric glands

Nitric oxide is generated through the oxidative metabolism of one of the guanidino nitrogens of L-arginine and L-citrulline is concomitantly produced as a byproduct. In the present study, glandular suspension at pH 7.4 was incubated for 30 min at 37℃ under oyxgen supply and [^{14}C]-L-arginine and various concentrations of dibutyryl cAMP(10^{-6} M -10^{-3} M) were added with calcium and NADPH for the activation of NO synthase. After 1 h of oxygenation, NO synthase activity was determined by percent conversion to [^{14}C]-L-citrulline from [^{14}C]-L-arginine, substrate of NO synthase. As shown in Fig. 3, there was no correlation between treatment of dibutyryl cAMP and NO synthase activities of gastric glands ($P>0.05$). NO synthase activities of dibutyryl cAMP-treated gastric glands were 107~111% of non-treated control (14.4±1.0% conversion) and there was no significant difference between dibutyryl cAMP-treated gastric glands and control in NO synthase activities.

Effects of hypoxia/reoxygenation and acidosis on NO synthase activity of gastric glands

Since dibutyryl cAMP did not stimulate NO synthase activities of gastric glands, changes of NO synthase activities were monitored in unstimulated gastric glands after hypoxia/reoxygenation and setting of acidosis, changing pH of incubation medium from pH 7.4 to pH 6.0 and pH 4.0 (Fig. 4). NO synthase activities, calculated as dpm of [^{14}C]-L-citrulline/dpm of [^{14}C]-L-arginine×100, of normoxic gastric glands at pH 7.4, 6.0 and 4.0 were 13.97±1.7%, 13.10±0.35% and 4.45±0.22%, respectively. This result was in accordance with suppressive effect of acidosis on acid secretion (Fig. 2.), which confirms that acidosis certainly inhibit glandular function with respect to both NO synthase activity and acid se-

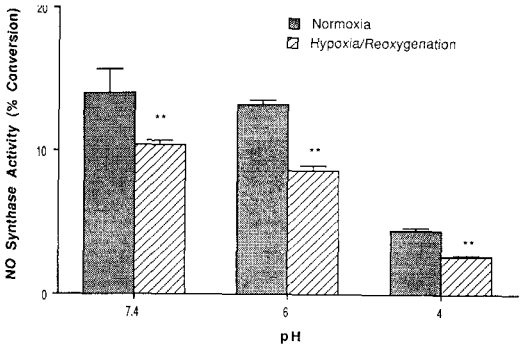

Fig. 4. Effects of hypoxia/reoxygenation and acidosis on NO synthase activity of gastric glands. Each bar represents mean±S.E.M. (n=5). ** P<0.01; compared with normoxic basal gastric glands.

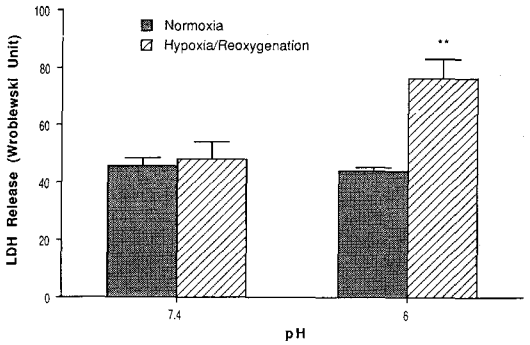

Fig. 6. Effects of hypoxia/reoxygenation and acidosis on cell viability of gastric glands. Each bar represents mean±S.E.M. (n=5). ** P<0.01; compared with normoxic gastric glands.

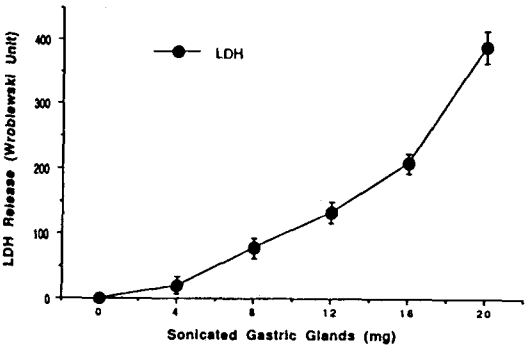

Fig. 5. LDH release as an index of cell viability. To test cell viability, various proportions of sonicated glands and freshly prepared glands were mixed (total 20 mg weight) and LDH release was determined. LDH release increased with proportions of sonicated gastric glands. Each point represents mean±S.E.M. (n=5).

cretion. Hypoxia/reoxygenation significantly inhibited NO synthase activities (P<0.01) at pH 7.4, 6.0 and 4.0, which were 10.37±0.35%, 8.62±0.3% and 2.62±0.09%.

Effects of hypoxia/reoxygenation and acidosis on cell viability of gastric glands

Isolated gastric glands were suspended at pH 7.4, 0.5<.0 and 4.0 for 30 min at 37℃± under 100% N_2 or 100% O_2. Further incubation was carried out under continuous oxygen supply for 1 h, finally which made hypoxia/reoxygenation or normoxic condition. To determine that release of LDH (lactic dehydrogenase) is an useful index of glandular cell viability, various proportions of sonicated glands and freshly prepared glands were mixed (total weight, 20 mg) and LDH release was measured (Fig. 5). LDH release was increased with proportions of sonicated gastric glands and LDH assay was used as an index of cell viability for the studies on hypoxia/reoxygenation and acidosis (Fig. 6). At pH 7.4, LDH release of normoxic gastric glands and hypoxic/reoxygenated gastric glands were 45.5±3 and 48.1±6 IU, which were similar to that of normoxic gastric glands incubated at pH 6.0 (44.1±1 IU). However, LDH release into medium of hypoxic/reoxygenated gastric glands incubated at pH 6.0 was 76.4±7 IU. This supports that hypoxia/reoxygenation in combination with acidosis severely damaged the cell and suppressed cellular function, monitored by inhibited acid secretion and NO synthase activity in present study. LDH activity in medium of gastric glands incubated at pH 4.0 was under control value (5.7±2.4 IU). This result means that LDH may be decomposed at highly acidic condition and measurement of LDH release is not a good index of cell viability in the studies related to acidosis.

DISCUSSION

Acid secretion by the stomach is initiated by specific receptors on the surface of parietal cells. The main pathway stimulating acid secretion is triggered by the binding of histamine to H_2-histamine receptor leading to G protein-mediated increase in adenylate cyclase activity. Activation of adenylate cyclase results in accumulation of adenosine 3', 5'-cyclic monophosphate (cAMP), which stimulates cAMP-depedent protein kinase a (PKA). PKA enhances pumping of protons by K^+-H^+-ATPase (Chew, 1991). In the present study, dibutyryl cAMP stimulated acid secretion dose-dependently and 10^{-3} M of dibutyryl cAMP increased acid secretion to 250% of unstimulated control, which is in agreement with the result of Wallmark *et al.* (1985).

The stimulatory effect of dibutyryl cAMP on acid secretion was significantly inhibited by both hypoxia/reoxygenation and acidosis. Yanaka *et al.* (1992) reported that 30 min of hypoxia changed the morphology of oxyntic cells from the secretory to the nonsecretory state without recognizable cytopathology and 1 h of hypoxia with 100% N_2 resulted in complete inhibition of H^+ secretion in frog gastric mucosa at pH 7.2 *in vitro*. They also suggested that basolateral acidosis exaggerates hypoxic injury of oxyntic cells. Besides overproduction of H^+ within cytoplasm as a result of anaerobiosis in hypoxic tissues (Hochachka and Mommsen, 1983), acidosis activates the release of oxygen free redicals (Kubes *et al.*, 1990), which also produced by reperfusion (Parks and Granger, 1986) and accentuate cell injury during hypoxia/reperfusion. This is proven by increased mucosal injury in animals subjected to 3 h of ischemia and 1 h of reperfusion compared with those subjected to 4 h of ischemia alone in small intestine (Parks and Granger, 1986b). Present results show that acid secretion was suppressed by hypoxia/reoxygenation both in unstimulated and stimulated gastric glands at pH 7.4 and pH 6.0 and highly acidic environment (pH 4.0) completely abolished secretory function, which was presented by little response to dibutyryl cAMP.

Ca^{++}-dependent constitutive NO synthase was found in gastric mucous cell fraction (Brown *et al.*, 1992), indicating the role of NO in regulation of mucosal integrity or secretion. In present study, hypoxia/reoxygenation decreased NO synthase activity at pH 7.4, 6.0 and 4.0 and acidosis exaggerated NO synthase activity in basal gastric glands. Since NO appears to have a protective role in gastric mucosa by modulating mucosal integrity (Pique *et al.*, 1989), regulating blood flow (Pique *et al.*, 1989), secretion of mucus or bicarbonate (Flemstrom, 1987), reduced NO production due to gastric hypoxia/reoxygenation and acidosis may accentuate gastric mucosal injury. In addition, NO was reported to scavenge oxygen free radical (Ignarro, 1989; Ialenti *et al.*, 1992; Gryglewski *et al.*, 1986) and reactive oxygen metabolites produced by hypoxia/reoxygenation could more seriously damage gastric mucosa. The reason why NO synthase activity is reduced by hypoxia/reoxygenation and acidosis has not been clarified but it may be contributed by possible destruction of this enzyme or reduced general cellular function due to reactive oxygen metabolites produced or loss of functional integrity by hypoxia/reoxygenation and acidosis. From present study, we found that there seems no correlation between acid secretion stimulated by dibutyryl cAMP and NO synthase activity in basal gastric glands. There are controversies in the role of NO for gastric acid secretion. Potent NO synthase inhibitor, L-N^G-nitro-L-arginine methyl ester prevented the acute inhibition by endotoxin of gastric acid responses to i.v. bolus administration of pentagastrin (Martinez-Cuesta *et al.*, 1992), suggesting involvement of inducible NO synthase in inhibition of acid secretion by unknown mechanism. Takeuchi *et al.*, (1992) reported that NO may be involved as the endogenous inhibitor in the regulation of HCO_3^- secretion, considered as one of the defensive factors in the stomach (Flemstrom and Turnberg, 1984). Recent studies showed that NO could be involved in the protective actions of some antiulcer drugs such as carbenoxolone and sucralfate (Dembinska-Kiec *et al.*, 1991; Konturek *et al.*, 1993). On the other hand, L-arginine, the precursor of NO, is reported to act on the stomach similar to mild irritants and protect mucosa possibly by generating endogenous prostaglandins, but not through the NO-mediated pathway (Takeuchi *et al.*, 1993). However, all these

studies are trying to investigate the role of NO in some pathologic state of stomach by using endotoxin (Martinez-Cuesta *et al.*, 1992), HCl (Takeuchi *et al.*, 1993), ethanol (Takeuchin *et al.*, 1994), or NaCl (Konturek *et al.*, 1993). The importance of the present finding is that there is no correlation of acid secretion stimulated by dibutyryl cAMP and NO synthase activity in basal state of gastric glands.

Finally, the authors tried to determine whether reduced acid secretion and NO synthase activity in hypoxia/reoxygenation and acidosis is related to cell viability and found that hypoxia/reoxygenation had no effect on cell viability, measured as LDH release at pH 7.4 and even at pH 7.4 and 6.0, LDH release was not significantly different in normoxic gastric glands. However, hypoxia/reoxygenation at pH 6.0 significantly reduced cell viability, which might be related to cessation of acid secretion and NO synthase activity.

The present study shows that hypoxia/reoxygenation suppresses acid secretion both in unstimulated and dibutyryl cAMP-stimulated gastric glands, which is exaggerated by acidosis. Constitutive NO synthase, not responded to dibutyryl cAMP, is also inhibited by both hypoxia/reoxygenation and acidosis although the mechanisms responsible for this phenomenon have not been fully evaluated yet. Further studies to elucidate the role of NO in acid secretion in normal and pathologic state of gastric mucosa, NO synthase inhibitors, NO donors, stimulants and inhibitors of acid secretion should be applied in gastric glands.

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=국문초록=

Hypoxia/Reoxygenation과 Acidosis가 위선세포에서 위산분비와 NO Synthase 활성에 미치는 영향

연세대학교 의과대학 약리학교실

김 혜 영 · 김 경 환

NO의 위산분비에 대한 작용을 규명하기 위하여 분리한 토끼위선세포에서 hypoxia/reoxygenation과 acidosis후 위산분비와 NO synthase 활성을 측정하였다. 분리한 위선세포에 30분의 hypoxia와 1시간의 reoxygenation을 주었으며, acidosis를 위하여 배지의 pH를 6.0과 4.0으로 변화시켜 실험하였다. 위산분비는 위선세포 내와 외의 [^{14}C]-aminopyrine 축적비율로 측정하였으며, NO synthase 활성은 NO의 전구물질인 [^{14}C]-L-arginine으로 부터 [^{14}C]-L-citrulline으로의 전환율로 결정하였다. 결과로서 dibutyryl cAMP는 농도 의존적으로 위산분비를 촉진시켰으나 NO synthase 활성엔 영향을 주지 않았다. Hypoxia/reoxygenation은 기초 및 자극 위산분비를 억제하였으며 acidosis에 의해 위산분비억제는 더욱 심화되었다. Constitutive NO synthase 활성 역시 hypoxia/reoxygenation과 acidosis에 의해 억제되었다. 결론적으로 hypoxia/reoxygenation과 acidosis 같은 위점막의 병적상태는 위산분비와 NO 유리를 모두 억제하나, 기초상태의 위선에서 dibutyryl cAMP에 의한 위산분비 촉진에 대한 NO의 직접적인 작용은 확인되지 않았다.