THE EFFECTS OF AMMONIA ON PANCREATIC ENZYME SECRETION IN VIVO AND IN VITRO

Background: Recent studies clearly demonstrate that Helicobacter pylori (H. pylori) infection of the stomach causes persistent elevation of ammonia (NH₃) in gastric juice leading to hypergastrinemia and enhanced pancreatic enzyme secretion. Methods: The aim of this study is to evaluate the influence of NH₄OH on plasma gastrin level and exocrine pancreatic secretion in vivo in conscious dogs equipped with chronic pancreatic fistulas and on secretory activity of in vitro isolated acini obtained from the rat pancreas by collagenase digestion. The effects of NH₄OH on amylase release from pancreatic acini were compared with those produced by simple alkalinization of these acini with NaOH. Results: NH₄OH given intraduodenally (i.d.) in increasing concentrations (0.5, 1.0, 2.0, 4.0, or 8.0 mM/L) resulted in an increase of pancreatic protein output, reaching respectively 9%, 10%, 19%, 16% and 17% of caerulein maximum in these animals and in a marked increase in plasma gastrin level. NH₄OH (8.0 mM/L, i.d.) given during intravenous (i.v.) infusion of secretin (50 pmol/kg.h) and cholecystokinin (50 pmol/kg.h) reduced the HCO₃⁻ and protein outputs by 35% and 37% respectively, as compared to control obtained with infusion of secretin plus cholecystokinin alone. When pancreatic secretion was stimulated by ordinary feeding the same amount of NH₄OH administered i.d. decreased the HCO₃⁻ and protein responses by 78% and 47% respectively, and had no significant effect on postprandial plasma gastrin. In isolated pancreatic acini, increasing concentrations of NH₄OH (10⁻⁷–10⁻⁴ M) produced a concentration-dependent stimulation of amylase release, reaching about 43% of caerulein-induced maximum. When various concentrations of NH₄OH were added to submaximal concentration of caerulein (10⁻¹² M) or urecholine (10⁻⁷ M), the enzyme secretion was reduced at a dose 10⁻⁵ M of NH₄OH by 38% or 40%, respectively. Simple alkalinization with NaOH of the incubation medium up to pH 8.5 markedly stimulated basal amylase secretion from isolated pancreatic acini, whereas the secretory response of these acini to pancreatic secretagogues was significantly diminished by about 30%. LDH release into the incubation medium was not significantly changed in all tests indicating that NH₄OH did not produce any apparent damage of pancreatic acini and this was confirmed by histological examination of these acini. Conclusions: 1. NH₄OH affects basal and stimulated pancreatic secretion. 2. The excessive release of gastrin may be responsible for the stimulation of basal pancreatic enzyme secretion in conscious animals, and 3. The inhibitory effects of NH₄OH on stimulated secretion might be mediated, at least in part, by its direct action on the isolated pancreatic acini possibly due to the alkalinization of these acini.

Key words: ammonia, pancreatic acini, pancreatic secretion, plasma gastrin, alkalinization.
INTRODUCTION

Helicobacter pylori (H. pylori) is now recognized as the most important factor in the pathogenesis of chronic gastritis and duodenal ulcer disease as well as gastric cancer (1—4). The toxic effect of H. pylori on gastric mucosa has been attributed, at least in part, to the synthesis of excessive amounts of ammonia (NH₄OH) produced by this germ due to its high urease activity (5, 6). NH₄OH protects H. pylori from injury by gastric H⁺ through raising pH and alkalization of gastric milieu around the bacteria (3—8). This alkalization affects the physiological mechanisms of regulation of gastric acid secretion and gastric release. H. pylori infection of the stomach is accompanied by hypergastrinemia partly due to the profound deficiency in local somatostatin release from antral D cells in the close vicinity of G cells (9—12). Gastrin as well as somatostatin are involved in the regulation of exocrine pancreatic secretion and impairment in the release of these hormones might affect pancreatic secretion of enzymes (13). Recent report demonstrates that subjects with H. pylori infection which show higher plasma gastrin concentration, exhibit high interdigestive, but not postprandial, pancreatic amylase secretion (14). This enhancement of pancreatic secretory function could be related to the hypergastrinemia produced by H. pylori infection, particularly that gastrin was shown to stimulate directly the pancreatic enzyme secretion and specific CCKₐ/gastrin receptors for gastrin were characterized on pancreatic acinar cells (15). On the other hand alkalization caused by larger amounts of NH₄OH by itself could change the secretory ability of pancreatic cells, but this possibility has not been explored before (16).

This study was undertaken to assess the effect of NH₄OH on basal and postprandial pancreatic secretion, as well as on plasma gastrin level in dogs with chronic pancreatic fistula. The direct secretory effects of NH₄OH on the pancreas were determined using pancreatic acini isolated from the rat pancreas. The effects of NH₄OH on amylase release from dispersed pancreatic acini under basal conditions and after stimulation with pancreatic secretagogues were compared with those obtained with simple alkalization by NaOH added to the medium bathing these acini.

MATERIALS AND METHODS

Following items were purchased: ammonia (NH₄OH) and ammonium chloride (NH₄Cl) from CIECH, Polish Biochemical Co., Gliswic, Poland, caerulein (Takus) from Pharmacia GmbH, Erlangen, Germany, urecholine, bombesin and trypsin inhibitor from Sigma Chemical Co. St. Louis MO, USA, essential and nonessential amino acid mixture from SEVRA Feinbiochemica GmbH, Heidelberg, Germany, purified collagenase from Worthington Biochemical Co., Freehold, NJ, USA, ¹²⁵I Gastrin tracer from Du Pont NEN, anti-gastrin antibodies used for gastrin radioimmunoassay were kindly gifted by prof. J. Rechfeld, Denmark, and LDH colorimetric slides were from KODAK EKTACHEM.
Pancreatic secretion in vivo

Pancreatic secretory studies were carried out on six mongrel dogs, weighing 18—22 kg, prepared surgically with pancreatic fistulas (PF) (17, 18). Briefly, to form the PF, the duodenal pouch containing the exit of the major pancreatic duct was fashioned from the middle portion of duodenum containing the entrance of major pancreatic duct, while minor pancreatic duct was ligated. The lateral limb of the metal cannula of PF was inserted into this pouch whereas the main limb of the cannula was placed in the distal duodenum about 3 cm beyond the duodeno-duodenostomy (17, 18). During the experiment, the hollowed obturator was inserted into the main limb of the cannula to collect the pancreatic juice not contaminated by the duodenal content and/or to instill intraduodenally the tested solutions of NH₄OH or NH₄Cl. The operation was performed under fully aseptic conditions and anesthesia induced by i.v. injection of 50 mg/kg pentobarbitone sodium (Polfa, Poland). A tracheal tube was inserted in each animal and a Harvard respirator was used to maintain intermittent positive pressure respiration when necessary. During recovery, animals' hydration was ensured in first 2 days after surgery by the s.c. administration of 11 of 0.15 M NaCl per day. Then, liquid meal was given for one week and then normal diet. Postoperatively, the dogs lost 2—4 kg of he body weight during the first but then regained their normal weight during the subsequent 2—3 weeks. They were allowed to recover fully over a period of about 3 months and remained in excellent health throughout the examination period. The studies reported here started about 6 mo after surgery and were carried out for about 9 mo.

Before each experiment, the food but not water was withheld for at least 18 h. Secretion from PF was collected continuously in 15 min aliquots. The volume was recorded to the nearest 0.1 ml. Pancreatic HCO₃⁻ protein concentrations and outputs were measured in each collected sample of pancreatic juice and expressed in 15 min outputs. Several tests were performed on each animal and the experiments on the same animal were carried out not often than once per week. In tests with secretory stimulation, the pancreatic secretion was first collected under basal conditions and then after the induction of pancreatic secretion with exogenous hormones [secretin plus cholecystokinin-8 (CCK) or with ordinary meat feeding. In separate experiments each animal was tested using graded doses of i.v. caerulein infusion (0.5—64 μg/kg h) to induce maximal protein response (caerulein maximum) in these dogs.

In tests with basal secretion, after 30 min of collection of pancreatic juice, NH₄OH or NH₄Cl (used as control solution) was given intraduodenally in gradullay increasing concentrations (0.5, 1.0, 2.0, 4.0, 8.0 mM/L) but at a constant rate of 80 ml/h. Each concentration of tested solution was instilled for 30 min and then doubled during the same experiment. In tests with feeding each dog was offered a single meal consisting of 500 g of homogenized cooked ground beef that was usually completely consumed within few minutes. When the secretory response to the meal reached a well sustained plateau, NH₄OH or NH₄Cl was instilled i.d. in a constant concentration (8 mM/L) and constant volume (80 ml/h). In tests with i.v. infusion of secretin (50 pmol/kg h) plus CCK (50 pmol/kg h), NH₄OH or NH₄Cl at the same concentration and rate (8 mM/L, 80 ml/h) was instilled i.d. after 45 min of secretory hormonal and meal stimulation.

Determination of plasma gastrin concentration:

In some tests blood samples were taken from the peripheral vein at 30-min intervals for the determination of plasma gastrin using radioimmunoassay as described previously (18).
Pancreatic secretion in vitro

Pancreatic secretion of amylase was measured using dispersed acini obtained from the pancreas of 24 h fasted anesthetized rats, and prepared by digestion with purified collagenase (CLSPA 500 U/ml) as described previously (19, 20). Pancreatic acini were then suspended in fresh medium of pH 7.4 containing: 24.5 mM HEPES, 98.0 mM NaCl, 4.0 mM KCl, 11.7 mM KH₂PO₄, 0.3 mM CaCl₂, 1.0 mM MgCl₂, 5.0 mM glucose, 1% (wt/vol) essential and nonessential amino-acid mixture, 1% bovine serum albumin and 0.01% trypsin inhibitor. Incubation medium was saturated with oxygen and maintained at 37°C in a shaking bath.

Amylase release was determined by incubation of acinar suspensions for 30 min in the presence of increasing concentrations of caerulein (10⁻¹³–10⁻⁶ M) or urecholine (10⁻⁷–10⁻³ M). Various concentrations of NH₄OH or NH₄Cl (10⁻⁶–10⁻₄ M) alone or in the combination with submaximal concentrations of caerulein (10⁻¹² M) or urecholine (10⁻⁵ M) were then added to the incubation medium of isolated pancreatic acini. To determine the effect of alkalization on amylase release from isolated pancreatic acini the pH of the incubation medium was increased up to 8.5 by addition of 1% solution of NaOH to the incubation medium with these acini. After 30 min of incubation, the acinar suspension was centrifuged at 1000 × g for 5 min and the supernatant was separated from the pellet. The amylase content of supernatant and dissolved pellet was determined separately using the method of Bernfeld (21). Amylase secretion was expressed as the percentage increase over basal value. The unstimulated amylase release during the entire experimental period (30 min) was determined and taken as the control value.

To determine the integrity of isolated pancreatic acini, the LDH release from these acini was measured using photometric method and EKTACHEM slides. The isolated acini were incubated for 30 min in the presence of various concentration of NH₄OH (10⁻⁷–10⁻⁴ M), NH₄Cl (10⁻⁷–10⁻⁴ M), caerulein (10⁻¹¹ M), urecholine (10⁻⁴ M). In addition, the acini were submitted to 30 min incubation in the medium at pH 8.0 or pH 8.5 and used to examine the release of LDH. Furthermore, the acini exposed to NH₄OH or NaOH were submitted to morphological examination under the light microscope.

Statistical evaluation:

Results are expressed as means ± SEM. The significance of the difference between means was evaluated using a two-way analysis for repeated measures (two-way ANOVA) followed by Duncan’s test with a level of confidence at p < 0.05.

RESULTS

Pancreatic secretion in vivo:

Basal pancreatic protein secretion showed characteristic periodic fluctuations around the average of about 20 ± 1.8 mg/15 min. NH₄OH given i.d. at the concentration of (0.5, 1.0, 2.0, 4.0 or 8.0 mM/L) resulted in an increase in pancreatic protein output, reaching respectively; 9, 10, 19, 16 and 17% of the caerulein maximum (765 ± 90 mg/15) in these animals and resulted also in a strong increase in plasma gastrin level (Fig. 1). In separate tests the maximal protein response was tested using i.v. infusion of graded doses of caerulein
(0.5–64 μg/kg-h), each dose being infused for 30 min and then doubled. The highest 30 min protein output attained by i.v. infusion of caerulein was considered as secretory maximum and it was reached at a dose of 32 μg/kg-h of caerulein. After a meat meal, the pancreatic protein and HCO$_3^-$ secretion reached the level attaining 52% and 60% of the respective maximal response to exogenous caerulein (32 μg/kg-h) and secretin (164 pM/k-h) (Figs 2 and 3). Postprandial gastrin level reached 161.2 ± 18.3 pM. Instillation of NH$_4$OH (80 mM/L, 80 ml/h) into the duodenum decreased the pancreatic protein and HCO$_3^-$ responses by 47 and 78%, respectively (Figs 2 and 3), but had no significant effect on plasma gastrin level. (57.4 ± 19.5 pM).

The pancreatic response to the combination of secretion plus CCK, each infused in constant dose (50 pmol/kg-h) was characterized by the copious volume flow and well-sustained secretion of HCO$_3^-$ and protein. NH$_4$OH (80 mM/L, 80 ml/h) instilled i.d. during i.v. infusion of these stimulants (secretin plus CCK) reduced the protein and HCO$_3^-$ outputs by 37 and 35%, respectively, as compared to the control response obtained with each of these stimulants combined with i.d. instillation of vehicle saline (80 ml/h) (Figs 4 and 5). Instillation into duodenum of NH$_4$Cl (80 mM/L, 80 ml/h) failed to affect significantly the pancreatic response to feeding was well as that stimulated by i.v. infusion of secretion plus CCK. Similar effects were recorded following i.d. application of saline at the same rate (80 ml/h).

Pancreatic secretion in vitro:

Spontaneous amylase release from the isolated pancreatic acini incubated at 37°C for 30 min in a shaking bath averaged about 3.0 ± 0.4%. Incubation of these acini in the presence of various concentrations of NH$_4$OH (10$^{-7}$ – 10$^{-4}$ M) resulted in the significant increase of amylase release. This increase started at the concentration of 10$^{-6}$ of NH$_4$OH and reached about 7.4 ± 1.4% at 10$^{-4}$ M of NH$_4$OH, that was about 43% of maximal amylase release obtained with caerulein at 10$^{-11}$ M (Fig. 6). Addition to the medium of NH$_4$Cl at the same concentration range to that of NH$_4$OH did not produce any significant change of enzyme secretion from isolated pancreatic acini (Fig. 6).

The comparison of the effects of NH$_4$OH and simple alkalinization by adding NaOH to incubation medium on amylase release from isolated pancreatic acini under resting conditions is shown on Fig. 7. As in studies mentioned above the addition to the incubation medium of NH$_4$OH at 10$^{-6}$ M and 10$^{-5}$ M raising the pH of medium from pH 7.8 to pH 8.1 caused usual significant and concentration-dependent increments in the unstimulated amylase release from the dispersed pancreatic acini (Figs 6 and 7). However similar increase of secretory activity of these acini was observed with simple elevation of medium pH by adding NaOH raising the pH up to 8.0 and (8.5) (Fig. 7).
Fig. 1. Effects of graded concentrations of NH₄OH or NH₄Cl on plasma gastrin level and basal pancreatic protein output in dogs with chronic pancreatic fistulas. Asterisk indicates significant (p<0.05) increase above the basal enzyme secretion. Means ± SEM of 3–6 separate experiments.

Fig. 2. Effects of graded concentrations of NH₄OH or NH₄Cl on postprandial pancreatic protein secretion in dogs with chronic pancreatic fistulas. Asterisk indicates significant (p<0.05) decrease below the value obtained with feeding alone. Means ± SEM of 3–6 separate experiments.
Fig. 3. Effects of graded concentrations of NH₄Cl on postprandial pancreatic bicarbonate secretion in dogs with chronic pancreatic fistulas. Asterisk indicates significant (p < 0.05) decrease below the value obtained with feeding alone. Means±SEM of 3—6 separate experiments.

Fig. 4. Effects of graded concentrations of NH₄OH or NH₄Cl on pancreatic protein secretion stimulated by intravenous administration of CCK + secretin in dogs with chronic pancreatic fistulas. Asterisk indicates significant (p < 0.05) decrease below the enzyme secretion stimulated by secretagogues alone. Means±SEM of 3—6 separate experiments.
Fig. 5. Effects of graded concentrations of NH₄OH or NH₄Cl on pancreatic bicarbonate secretion stimulated by intravenous administration of CCK + secretin in dogs with chronic pancreatic fistulas. Asterisk indicates significant (p < 0.05) decrease below the secretion stimulated by secretagogues alone. Means ± SEM of 3—6 separate experiments.

Fig. 6. Effects of increasing concentrations of NH₄OH and NH₄Cl on amylase release from isolated pancreatic acini under resting conditions. Column on the right shows maximal amylase response to caerulein from these acini. Asterisk indicates significant (p < 0.05) increase above the control value obtained in tests without the addition of NH₄OH or NH₄Cl. Mean ± SEM of results from 6 separate experiments.
Incubation of isolated pancreatic acini in the presence of $10^{-12}$ M of caerulein, or in the presence of $10^{-5}$ M of urecholine, that was shown in our previous experiments (20) to produce submaximal stimulation of amylase release, resulted in an increase of enzyme secretion up to $11.3 \pm 1.0\%$ and $11.8 \pm 0.7\%$, respectively (Fig. 8, Table I). Addition of gradually increasing concentrations of NH$_4$OH ($10^{-7} - 10^{-5}$ M) to the incubation medium of isolated pancreatic acini increased gradually the pH of this medium (from pH 7.6 at $10^{-7}$ M NH$_4$OH to pH 8.1 at $10^{-5}$ M of NH$_4$OH) and resulted in a concentration-dependent reduction in this caerulein- or urecholine-stimulated amylase release (Fig. 8). When isolated pancreatic acini were stimulated with caerulein at $10^{-12}$ M or urecholine at $10^{-5}$ in the presence of NH$_4$Cl (at $10^{-7}$ to $10^{-5}$ M) the amylase level was not significantly different from this stimulated by pancreatic secretagogues alone (Table I).
Fig. 8. Effect of various concentrations of NH₄OH on caerulein- or urecholine-induced amylase release from isolated pancreatic acini. Asterisk indicates significant (p < 0.05) decrease below the value obtained with secretagogue alone. C = control, obtained by the incubation of the acini at pH 7.4, without the addition of secretagogues or NH₄OH. Means ± SEM of 6 separate preparations of the acini.

Table 1. The effect of various concentrations of NH₄Cl on caerulein- or urecholine-induced amylase release from isolated pancreatic acini. Means ± SEM of 6 separate tests.

<table>
<thead>
<tr>
<th>Amylase release (% of total)</th>
<th>alone</th>
<th>+NH₄Cl 10⁻⁷ M</th>
<th>+CH₄Cl 10⁻⁶ M</th>
<th>+NH₄Cl 10⁻⁵ M</th>
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<tbody>
<tr>
<td>caerulein 10⁻¹² M</td>
<td>11.3 ± 1.0</td>
<td>11.8 ± 2.0</td>
<td>10.3 ± 1.3</td>
<td>10.0 ± 0.7</td>
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<tr>
<td>urecholine 10⁻⁵ M</td>
<td>11.8 ± 0.7</td>
<td>10.8 ± 0.5</td>
<td>12.0 ± 0.8</td>
<td>9.7 ± 1.3</td>
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</tbody>
</table>

The effects of simple alkalization with NaOH of incubation medium up to pH 8.0 or up to pH 8.6 on amylase release induced by caerulein or urecholine are shown on Fig. 9. Both caerulein- and urecholine-induced amylase release from isolated pancreatic acini were slightly reduced when alkalization of this medium by NaOH rose to pH 8.5.
Fig. 9. Effect of alkalization (pH 8.0 and pH 8.5) on amylase release from isolated pancreatic acini stimulated by submaximal doses of caerulein- or urecholine. Asterisk indicates significant (p < 0.05) decrease below the level obtained with secretagogue alone. Means ± SEM of 6 separate experiments.

Fig. 10. Pancreatic amylase dose-response curves to increasing concentrations of urecholine, obtained by the incubation of the acini in the medium at pH 7.4, pH 8.0 or pH 8.5. Asterisk indicates significant (p < 0.05) decrease below the value obtained with incubation of the acini with urecholine at pH 7.4. C = unstimulated control. Means ± SEM of 6 separate test.
The effect of alkalinization medium on amylase dose-response curve for urecholine is shown on Fig. 10. Urecholine added in increasing concentration ($10^{-7} - 10^{-3}$ M) to the incubation medium maintained at pH 7.4 produced concentration-dependent stimulation of amylase release, reaching a maximum at $10^{-4}$ M and then declining with further increase in urecholine concentration. Alkalinization of incubation medium to pH 8.5 resulted in the significant decrease of secretory responses to urecholine. The maximal secretory response obtained with concentration of $10^{-4}$ M of urecholine that achieved about $15.5 \pm 1.1\%$ of total amylase release at neutral pH 7.4, was reduced significantly to $12.8 \pm 1.0\%$ at pH 8.0 and to $9.5 \pm 0.5\%$ at pH 8.5 (Fig. 10).

Incubation of isolated pancreatic acini at pH 8.0 or pH 8.5 decreased the secretory response of these acini to increasing concentrations of caerulein in similar way to that observed in experiments with alkalinization of medium using urecholine to obtain dose response curve and these results are shown on Table 2.

*Table 2. Effect of alkalinization of incubation medium of isolated pancreatic acini on amylase dose-response curve to caerulein. Means $\pm$ SEM of 6 separate tests. Asterisk indicates significant decrease below the value obtained with incubation of isolated pancreatic acini at pH = 7.4.*

<table>
<thead>
<tr>
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<th>Amylase release (% of total)</th>
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<tr>
<td></td>
<td>pH = 7.4</td>
</tr>
<tr>
<td>Control</td>
<td>4.0 $\pm$ 0.5</td>
</tr>
<tr>
<td>caerulein $10^{-11}$ M</td>
<td>8.0 $\pm$ 0.5</td>
</tr>
<tr>
<td>caerulein $10^{-12}$ M</td>
<td>11.3 $\pm$ 1.0</td>
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<tr>
<td>caerulein $10^{-11}$ M</td>
<td>17.2 $\pm$ 1.5</td>
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<tr>
<td>caerulein $10^{-10}$ M</td>
<td>15.8 $\pm$ 0.5</td>
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The effects of increasing concentrations of NH$_4$OH and alkalinization with NaOH of incubation medium on LDH release and histology of acinar cells are shown on Table 3. None of concentrations of NH$_4$OH used in our experiments as well as increase of extracellular pH up to 8.0 and up to 8.5 produced any significant change of LDH release from isolated pancreatic acini or in histological appearance of acinar cells as compared to those obtained with pH 7.4. This lack of influence of NH$_4$OH or alkalinization with NaOH on LDH release and histology of dispersed pancreatic acini indicates that neither NH$_4$OH used at concentrations as high as $10^{-4}$ M nor the alkalinization of incubation medium up to pH 8.5 caused any apparent impairment of these acini.
Table 3. Effect of ammonia and pH on LDH level in incubation medium (U/l) (A) and on morphology of isolated pancreatic acini (B).

<table>
<thead>
<tr>
<th>C</th>
<th>pH = 7.4</th>
<th>NH₄OH</th>
<th>10⁻⁷ M NH₄OH</th>
<th>10⁻⁶ M NH₄OH</th>
<th>10⁻⁵ M NH₄OH</th>
<th>pH = 8.0</th>
<th>pH = 8.5</th>
<th>10⁻¹¹ M caerulein</th>
<th>10⁻⁴ M urecholine</th>
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<tr>
<td>194±9.3</td>
<td>185±10.0</td>
<td>200±8.5</td>
<td>176±12.0</td>
<td>205±16.4</td>
<td>191±10.8</td>
<td>172±10.1</td>
<td>168±25</td>
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<tr>
<th>C</th>
<th>pH = 7.4</th>
<th>NH₄OH</th>
<th>10⁻⁷ M NH₄OH</th>
<th>10⁻⁶ M NH₄OH</th>
<th>10⁻⁵ M NH₄OH</th>
<th>pH = 8.0</th>
<th>pH = 8.5</th>
<th>10⁻¹¹ M caerulein</th>
<th>10⁻⁴ M urecholine</th>
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<td>edema</td>
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<tr>
<td>0 → 3</td>
<td>0.2±0.1</td>
<td>0.0</td>
<td>0.2±0.1</td>
<td>0.3±0.2</td>
<td>0.3±0.14</td>
<td>0.25±0.05</td>
<td>0.2±0.05</td>
<td>0.0</td>
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<td>vacuolisation</td>
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<tr>
<td>0 → 3</td>
<td>0.1±0.05</td>
<td>0.0</td>
<td>0.1±0.005</td>
<td>0.3±0.11</td>
<td>0.0</td>
<td>0.2±0.01</td>
<td>0.3±0.20</td>
<td>0.28±0.15</td>
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DISCUSSION

This study provides an evidence that ammonia (NH₄OH), one of the major gastrotoxic factors produced by *H. pylori* urease, is able to affect pancreatic enzyme secretion *in vivo* in dogs with chronic pancreatic fistula and *in vitro* in dispersed pancreatic acini. It stimulates pancreatic enzyme secretion under basal conditions but suppresses the secretory responses to various secretagogues. In intact animals, the stimulation by NH₄OH in duodenum of pancreatic enzyme secretion in the dogs with chronic pancreatic fistula was accompanied by a significant rise in gastrin plasma level. To the contrary, pancreatic secretion stimulated by infusion of secretion plus CCK or by ordinary feeding, producing equal rate of pancreatic secretory response, was significantly reduced by ammonia despite the enhanced increment in plasma gastrin levels.

Direct exposition of dispersed pancreatic acini, obtained by collagenase digestion from the rat pancreas, to ammonia also resulted in a significant increase of unstimulated amylase release, whereas enzyme secretion induced by caerulein or urecholine was inhibited by this ammonia in a concentration-dependent manner. These direct effects of ammonia on amylase release from pancreatic acini could be related, at least in part, to the elevation of extracellular pH because simple alkalinization of incubation medium by adding NaOH produced similar changes of enzyme secretion. An enhancement of amylase release in response to ammonia or NaOH was not due to the damage of acinar cells as both the release of LDH and histological appearance of these cells were virtually unchanged.
NH₄OH is produced in excessive amounts in subjects with infection of gastroduodenal mucosa with *H. pylori* due to activation of bacterial urease and exerts the biological effect in both in vivo gastric mucosa and in vitro various cell cultures (6, 22–4). NH₄OH is easily diffusible compound so it is likely that certain amount of this product of *H. pylori* infection of gastric and duodenal mucosa (due to gastric metaplasia) may reach directly the pancreas. As was previously shown, the intestine is able to absorb large amounts of NH₄OH with subsequent rise of this product in the portal blood (25). Since the pancreas is capable to accumulate rapidly the large amount of NH₄OH from the circulation (26) it is reasonably to assume that NH₄OH produced by *H. pylori* in the stomach and in duodenum reaching the pancreas may affect its secretory functions. This is why, we undertook regulator studies both in intact animals with chronic pancreatic fistulas and in vitro using isolated pancreatic acini to check the influence of NH₄OH on in vivo pancreatic secretory functions under basal, hormone-stimulated or postprandial secretion.

Since ammonia in acidic gastric milieu is transformed into inactive NH₄Cl we also assessed the effects of this substance on pancreatic enzymes secretion and gastrin release. It was found that NH₄Cl was completely ineffective in experiments both in vivo pancreas and in vitro isolated pancreatic acini. NH₄OH is a weak base that dissociates to NH₄⁺ and OH⁻ leading to the alkalization of the medium of isolated pancreatic acini, whereas NH₄Cl does not produce any alkalization. The lack of effects of NH₄Cl in our experiments provides an evidence that NH₄OH but not NH₄Cl is responsible for the alterations in pancreatic secretion and gastrin release in response to ammonia.

The relation between the *H. pylori* infection of the stomach and pancreatic dysfunction has been studied during last years (14, 27, 28). Clinical data suggested an associated between *H. pylori* infection and pancreatic cancer (4). Recent report showed that vacuolating cytotoxin of *H. pylori* (Vac) is capable of inhibiting amylase release from isolated pancreatic acini evoked by CCK-8 or carbachol. It is not know, however, if Vac could exert its inhibitory influence in vivo (29). Our present observation, concerning the increase of basal pancreatic enzyme secretion in response to ammonia is in keeping with the recent observations in humans by Dominguez-Munoz *et al.* (14) who found an increased pancreatic enzyme secretion in patients infected with *H. pylori*. This effect was attributed to the increased gastrin and reduced somatostatin release in these subjects due to direct action of *H. pylori* and its toxins on the G-cells and D-cells in the antral mucosa (14). The results of present in vivo study on dogs with chronic pancreatic fistula show that duodenal instillation of NH₄OH, but not NH₄Cl, results in the significant augmentation of pancreatic protein output accompanied by a marked rise in plasma gastrin level. Gastrin is, however, unlikely to stimulate pancreatic exocrine secretion under
physiological conditions because the elevation of gastrin plasma level after meal is too low to activate CCK$_B$ receptors on pancreatic acinar cells (30). Infection of _H. pylori_ produced local alkalization of the antral mucosa leading to the increase of gastrin plasma level that was several times higher than that occurring under physiological conditions (9—11). This large amounts of gastrin in _H. pylori_ infected mucosa could be responsible, at least in part for the augmentation of pancreatic enzyme secretion under basal conditions.

In contrast to the effects of NH$_4$OH in fasted dogs, in experiments with the stimulated of pancreatic secretion by the combination of CCK plus secretion or by a meat meal the pancreatic secretory functions were reduced by NH$_4$OH. CCK either released by a meal or infused i.v. binds to CCK$_A$ receptors, whereas gastrin acts on CCK$_B$ receptor on pancreatic acinar cells. The affinity of receptor for CCK is several times higher than those for gastrin (15). Each of these two hormones when given separately, stimulates enzyme secretion from pancreatic acini, but in the presence of both; gastrin and CCK, the interaction of the receptors may lead to the inhibition of this secretion. As evidenced in our present _in vivo_ study, high amount of gastrin released by ammonia, together with CCK, given either i.v. or released by the food, resulted in the reduction in pancreatic enzyme secretion. Above results do not fully support those presented by Dominguez-Munoz et al. in showing that in patients with _H. pylori_ infection no significant differences in postprandial pancreatic secretion was found when compared to non-infected controls (14). In this study, however, no attempts were made to clarify whether removal of _H. pylori_ infection and the fall in NH$_4$OH production influence the pancreatic secretion.

The major finding of the present study is the observation that NH$_4$OH may directly affect enzyme secretion from isolated pancreatic acini. In our studies we used a wide range of NH$_4$OH concentrations, and even through the highest of these doses are unlikely to exist in the stomach or to reach the pancreas in the course of _H. pylori_ infection, some direct influence of NH$_4$OH on pancreatic secretory function in _H. pylori_ infected subjects cannot be excluded.

In our present study NH$_4$OH added to the incubation medium also produced dose-dependent stimulation of amylase release from isolated pancreatic acini under resting conditions, whereas enzyme secretion from these acini stimulated by exogenous secretagogues such as caerulein or urecholine was reduced by NH$_4$OH. Since NH$_4$OH added to the incubation medium produced the increase of pH of this medium, we compared the secretory effects of NH$_4$OH with those produced by simple alkalization of medium of pancreatic acini using NaOH. To our surprise the elevation of pH of pancreatic acini medium up to 8.0 and 8.5 mimicked the secretory changes produced by NH$_4$OH, resulting in an augmentation of basal amylase release from these acini and reduction in secretagogue-induced secretion. It is very likely that the secretory effects of NH$_4$OH on the acinar cells observed _in vitro_ could be
explained, at least in part, by simple elevation of extracellular pH of these acini by this substance.

Previous reports showed that amylase release stimulated by pancreatic secretagogues as caerulein or carbachol is accompanied by cytoplasmatic alkalinization of the acinar cells (16). This alkalinization of intracellular pH is believed to be a result of conformational change of an amiloride-sensitive $Na^+ - H^+$ exchanger caused by phosphorylation of this exchanger by a protein kinase C (31). Phosphorylation by protein kinase C is one of the events that occur after stimulation of pancreatic acini by carbachol or caerulein (32) and it is likely that both these pancreatic secretagogues elevate intracellular pH via activation of kinase C (33). It was observed that the elevation of pH in the incubation medium resulted in the same change of pH inside the pancreatic cell (16). Our present results clearly demonstrates that alkalinization of pancreatic acini could stimulate amylase release from these acini by itself and that $NH_4OH$ increased amylase release from pancreatic acini most likely via alkalinization of these acini. This enhancement of amylase release from isolated pancreatic acini in response to $NH_4OH$ or alkalinization does not result from any damage of acinar cells as evidenced by of any significant change in LDH release or histology of the acinar cells that were exposed to $NH_4OH$ or NaOH for 30 min (standart time of incubation) at the concentration used.

Our results from in vitro study on isolated pancreatic acini confirm and reinforce our observation in vivo concerning the inhibitory effect of $NH_4OH$ on stimulated pancreatic enzyme secretion either by exogenous hormones or by feeding. Amylase release from isolated pancreatic acini stimulated submaximally by caerulein or urecholine was inhibited by $NH_4OH$ in the concentration-dependent manner. Simple alkalinization of the incubation medium of isolated pancreatic acini with NaOH resulted in a decrease of secretagogue-induced amylase release from these acini thus both $NH_4OH$ and alkalinization of the pancreatic acini produced similar inhibitory effects on stimulated pancreatic acini. This is in keeping with previous findings in isolated pancreatic acini showing paralleled changes in amylase dose-response curve to caerulein and to alterations in intracellular pH in the acini (16). It is of interest that the highest elevation of cytoplasmatic pH in this study (16) was observed with the supramaxial concentrations if caerulein, which actually resulted in the decrease of enzyme secretion from pancreatic acini (16). In the rat pancreas, caerulein binds to the CCK$_A$ receptors on pancreatic acinar cells (33), which can be divided into two subclasses; first having a high affinity and a low capacity for CCK and another class with a low affinity and high capacity for CCK. Occupation of a high-affinity CCK receptors correlates with the upstroke of the dose response curve for stimulated enzyme secretion, while occupation of low affinity receptors is related to the downstroke of the dose response curve for stimulated enzyme secretion (33). In addition, the existence
of two subclasses of muscarinic receptors in the pancreas was demonstrated; one with high affinity for carbachol or urecholine and another with a low affinity for other muscarinic agent (33). It appears, that maximal elevation of cytoplasmic pH correlates with the occupation of low affinity receptors for CCK\textsubscript{A} and it is accompanied by a decrease of amylase release from these acini. It is very likely that alkalization of pancreatic acini resulted in the change of receptor affinity for pancreatic secretagogues; decreased the occupation of high affinity receptors and increased the occupation of low affinity receptors, leading to the diminution of secretory response of pancreatic acini to pancreatic secretagogues.

In summary ammonia, the main gastrototoxic factor in Helicobacter pylori infection, appears to influence pancreatic enzyme secretion directly, acting on pancreatic acinar cells, as well as indirectly by stimulating gastrin release. The effects of ammonia on the pancreatic acini seem to be related, at least in part, to the alkalization of the gland produced by NH\textsubscript{4}OH.

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