ACID BACK-DIFFUSION AND MUCOSAL H⁺ HANDLING IN THE RAT STOMACH UNDER NORMAL AND STRESS-INDUCED CONDITIONS

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Abstract—We determined acid back-diffusion and pepsin output simultaneously in vagotomized rats after instillation of HCl into the stomach under normal and stress-induced conditions. With exposure to 6 ml of 100 mM HCl, spontaneous acid back-diffusion increased with the duration of the experiment under both conditions, and the magnitude of the acid back-diffusion was decreased significantly by stress. There was no change in the output of pepsin. While disappearance of luminal acid caused by aspirin or taurocholic acid was not altered by stress, the pepsin output in response to H⁺ increased significantly in the stressed rats. With exposure to various concentrations of HCl for 3 hr, disappearance of the luminal acid increased linearly with the grade of HCl under both conditions. Except for the concentration of 300 mM, the magnitude of the acid back-diffusion was triple in the normal condition, and the ratio of pepsin output/net flux of H⁺ was significantly increased by stress. Thus, (1) spontaneous acid back-diffusion decreased with stress, while diffusion induced by chemical barrier breakers remained the same; (2) the action of H⁺ diffused back into the mucosa did not always parallel the amount of diffusion determined from the loss of H⁺ in the lumen; (3) intramucosal H⁺ may be largely dissipated in normal mucosa; and (4) the initiation or aggravation of drug-induced mucosal damages by stress may be related to insufficiency of the H⁺ dissipating mechanisms.

Back-diffusion of H⁺ into the gastric mucosa was proposed by Davenport to be an important etiological factor in the pathogenesis of gastric ulcers (1–3). Although a large amount of data supports this view (4, 5), there are compounds such as p-chloromercuribenzenesulphonate (PCMB) which cause a sizable acid back-diffusion but rarely provoke gross erosions (6). On the other hand, aspirin induced gastric erosions are greatly aggravated by stress, even though the magnitude of the acid back-diffusion does not significantly differ between normal and stressed rats (7). Therefore, increase in the rate of H⁺ permeation does not in itself lead to gastric damages, and the magnitude of the acid back-diffusion does not seem to be directly associated with development of the mucosal damages. So, changes in the mucosal environment as induced by stress or drugs seem to be more important than acid back-diffusion in the initiation or aggravation of gastric damages. Recent studies showed that mucosal blood flow or nutrient HC0₃⁻ may be a possible factor in the H⁺ translocating or neutralizing system in the gastric mucosa (8–10). However, there is apparent no quantitative data on the action of H⁺ diffused back into the mucosa under in vivo conditions. Davenport and Johnson reported that release of histamine and pepsin secretion occur as a consequence of acid back-
diffusion (11, 12). Since pepsin secretion in response to H+ is mediated through a cholinergic pathway, it is theoretically feasible to quantitate the action of H+ in the mucosa by measuring pepsin output during the course of acid back-diffusion (13). We have now calculated the amount of pepsin output due to H+ in the mucosa in order to evaluate changes in gastric mucosal susceptibility to H+ under conditions of induced stress and to determine whether such changes relate to the initiation or aggravation of drug-induced mucosal damages by stress.

Materials and Methods

Male Donryu rats (200 to 220 g) were deprived of food, but allowed free access to water for 18 hr before start of the experiments.

Operative procedure

Under ether anesthesia, the pylorus and the distal end of the esophagus were ligated (at this time, the esophagus was loosely ligated), and bilateral subdiaphragmatic vagotomy was performed. A polyethylene tube was inserted into the lumen of the stomach through the esophagus, then 8 ml of test solution was instilled, and 2 ml of the solution was saved for the analysis as an initial sample. The tube was removed and the esophagus was tightly ligated. Rats subjected to the above procedure were divided into two groups. In the stress group, the rats were kept in stress cages and subjected to water immersion at 23°C. In the normal group, the rats were placed in individual cages with a raised mesh bottom, at room temperature.

Experiment 1

Time course changes in acid back-diffusion and pepsin output: The acid solution used contained 100 mM HCl and 54 mM NaCl. Phosphate buffer (pH 7.0) served as the control solution. All the rats were killed under ether anesthesia; and in both groups, the total gastric contents were collected at 3, 7 and 20 hr time periods. After centrifugation, both initial and final samples were analyzed for volume and titrated with 0.1 N NaOH to pH 7.0 using an Automatic Titrator (Radiometer, Copenhagen). The concentration of Na+ was determined using a Hitachi flame photometer. The net fluxes of H+ and Na+ through the gastric mucosa were calculated as the difference between the product of the final volume and concentration and the product of the initial volume and concentration. Positive values indicate that the net flux was from the serosa to the lumen. Pepsin activities in both samples were determined according to the modified Anson's method (14), and the substantial output was calculated in the same way as the net fluxes of H+ and Na+. Basal pepsin output was also determined by instillation of 8 ml of phosphate buffer instead of the acid solution.

Experiment 2

Relationship of acid back-diffusion and pepsin output: Various concentrations of HCl (50, 100, 200 and 300 mM) were used as acid solutions. Osmolarity of the test solution was adjusted by adding appropriate concentrations of NaCl and mannitol, except in the 200 and 300 mM HCl solutions. Three hr after instillation of the test solution, the rats were killed, and the net flux of H+ and pepsin output was measured.

Experiment 3

Acid back-diffusion and pepsin output in response to aspirin or taurocholic acid: In the 3 hr experiment (Exp. 2), effects of aspirin and taurocholic acid were examined on the net flux of H+ and pepsin output. These drugs were dissolved in acid solution (100 mM HCl plus 54 mM NaCl) in the dose of 100 mg/kg or 300 mg/kg, respectively. After collecting the gastric contents, stomachs were examined under a dissecting microscope (x10), and the gastric lesions were counted. In case of the rats given aspirin, the amount of acid back-diffusion was also determined at 3, 7 and 20 hr under normal and stress...
conditions as described in Exp. 1. The net flux of H⁺ and pepsin output were measured in each test.

Drugs

Drugs used were acetylsalicylic acid (aspirin) (Sigma) and taurocholic acid Na (Nakarai).

Statistics

All data are presented as the mean±S.E. of the values determined from 8 to 10 rats. The level of significance was determined using the unpaired Student’s t-test. Values of P<0.05 were regarded as significant.

Results

1. Back-diffusion of H⁺ and pepsin output under normal and stress conditions: As shown in Fig. 1, the decrease of H⁺ and the increase of Na⁺ increased with time under both conditions. The degree of changes in both ions, however, was significantly different between normal and stressed rats. In stressed rats, the reduction of H⁺ and the increment of Na⁺ were about half the values observed in normal rats at the 3 or 7 hr time period; but at 20 hr, both fluxes were not significantly different between these two groups. In contrast to the spontaneous acid back-diffusion, when the mucosal barrier was disrupted with aspirin, the net flux of H⁺ and Na⁺ in the stressed rats was comparable to that found in the normal rats throughout the entire period of experiment.

Pepsin output in the vagotomized stomach was negligible by instillation of 6 ml of phosphate buffer (pH 7.0) throughout the experimental period. As evidenced from Fig. 2, pepsin output in the group subjected to the instillation of acid solution (100 mM HCl plus 54 mM NaCl) increased with time under both conditions, and this was parallel with the occurrence of acid back-diffusion (see
There was no significant difference in pepsin output in these two groups at any time period, although the ratio of pepsin output/the net flux of H+ was significantly lower in the normal rats (Fig. 3). Since it is evident from the results of phosphate buffer that the pepsin secretion in the vagotomized stomach was due to the H+ back-diffusion, the substantial amount of H+ diffused back into the gastric mucosa would be almost equivalent in both conditions, despite a marked difference in the magnitude of H+ loss in the lumen of the stomach.

2. Relationship between pepsin output and acid back-diffusion: To determine the relation between pepsin output and acid back-diffusion, both parameters were measured concomitantly with varying concentrations of HCl in the acid test solution at the 3 hr period. As shown in Fig. 4, the back-diffusion of H+ occurred in proportion to the grade of acid loading in both normal and stressed rats. In the former, the net flux of H+ usually increased linearly with the concentration of acid loaded; while in the stressed rats, a similar relation could be obtained over a limited range (50 mM to 200 mM). However, with 300 mM HCl, the amount of acid back-diffusion remarkably deviated from the line, probably due to mucosal damages caused by the hypertonicity of the acid solution. Actually, blood was detected in the gastric contents in the stressed rats when 300 mM HCl had been given. When pepsin output obtained simultaneously was plotted against the net flux of H+ diffused back into the mucosa (determined from the luminal loss of H+), it was clear that the output was strongly potentiated in response to H+ under the condition of stress. Figure 5 shows that an antagonistic action in the mucosa is operative against influxed H+ under the normal condition.

3. Effects of stress on H+ back-diffusion and pepsin output caused by aspirin or taurocholic acid: Table 1 shows the net flux
Fig. 5. Lineweaver-Burk plot analysis of pepsin output in response to H⁺ back-diffusion. Experimental procedures were outlined in Fig. 4-A and B. A least-squares linear regression analysis was performed; the correlation coefficient (r) being 0.9748 in the normal condition and 0.9519 in the stress condition. The maximal pepsin output is given by the reciprocal of the y-intercept. The value obtained from the reciprocal of the x-intercept indicates the amount of H⁺ required to stimulate 50% of the maximal pepsin output.

Table 1. Effects of stress on aspirin or taurocholic acid-induced H⁺ back-diffusion, pepsin output and gastric lesions in rats with vagotomized stomachs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of animals</th>
<th>H⁺ Back-diffusion (μEq/3 hr)</th>
<th>Pepsin output (mg/3 hr)</th>
<th>Ulcer index (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Acid solution</td>
<td>—</td>
<td>10</td>
<td>165.0±13.8</td>
<td>15.2±1.3</td>
<td>0</td>
</tr>
<tr>
<td>B. Aspirin (ASA)</td>
<td>100</td>
<td>10</td>
<td>327.5±10.8</td>
<td>24.6±1.2</td>
<td>17.0±3.2</td>
</tr>
<tr>
<td>C. Taurocholic acid (TCA)</td>
<td>300</td>
<td>10</td>
<td>361.0±18.1</td>
<td>14.8±1.9</td>
<td>0</td>
</tr>
<tr>
<td>Stress condition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Acid solution</td>
<td>—</td>
<td>10</td>
<td>46.5±12.1</td>
<td>19.1±2.3</td>
<td>0</td>
</tr>
<tr>
<td>E. ASA</td>
<td>100</td>
<td>10</td>
<td>325.4±14.4</td>
<td>30.6±1.1</td>
<td>39.0±4.3</td>
</tr>
<tr>
<td>F. TCA</td>
<td>300</td>
<td>10</td>
<td>386.9±7.5</td>
<td>19.5±1.0</td>
<td>18.3±2.1</td>
</tr>
</tbody>
</table>

Each drug was dissolved in acid solution (100 mM HCl plus 54 mM NaCl), and 6 ml of each solution was instilled by esophageal intubation. Animals were killed 3 hr after instillation of each solution.

Table 1 indicates that stress itself had no influence on acid back-diffusion caused by either drug. However, pepsin output was significantly increased by stress in both cases (24.4% for aspirin and 31.8% for taurocholic acid). Although pepsin output in taurocholic acid treated animals was underestimated...
because of the negative interaction between pepsin or substrate and this compound (7), gastric lesions developed in concert with the increase of pepsin output. There was no relationship between lesion index and the net flux of H⁺ (Fig. 6).

Fig. 6. Relationship between ulcer index and pepsin output or H⁺ back-diffusion. The amount of H⁺ back-diffusion was determined from the luminal loss of H⁺. The results shown in this Fig. were taken from Table 1. The reciprocal coefficient (r) is 0.5138 for the relation between ulcer index and H⁺ back-diffusion, and it is 0.9215 between the ulcer index and pepsin output.

Discussion

Pepsin output is induced by H⁺ diffused back into gastric mucosa as determined from the studies on the topical application of HCl or HCl plus barrier breakers in the stomach (12, 13). The mechanism of pepsin secretion in response to H⁺ is thought to involve cholinergic mediation through the Ach-receptor because pepsin secretion is inhibited by atropine or xylocaine (13). We applied this theory for a quantitative determination of the sensitivity of gastric mucosa against H⁺ diffused back into the mucosa. Participation of acid back-diffusion has been controversial in the etiology of ulceration induced by stress. Skillman and colleagues observed an increase in the luminal loss of H⁺ in the rabbit gastric mucosa under hemorrhagic shock (15), while Gerety and Guth found no increase in the rate of H⁺ permeation in cold restraint rats (16). Since normal gastric mucosa usually has a slight permeability to luminal H⁺, we examined the influence of stress on spontaneous (dependent chemical gradient of H⁺) and induced acid back-diffusion. Initially, the amount of acid which spontaneously diffuses back into the mucosa was significantly decreased by stress; however, the sizable acid back-diffusion caused by aspirin or taurocholic acid was not altered by subjecting the rats to water-immersion stress. These results are consistent with the finding by Gerety and Guth (16), and they indicate that acid back-diffusion does not increase under conditions of stress and that the spontaneous H⁺ permeation would be in marked contrast to the sizable permeation which accompanies chemical or mechanical disruption of the gastric mucosal barrier.

The output of pepsin which reflects the action of H⁺ in the mucosa was equivalent in response to the spontaneous acid back-diffusion in the stressed and normal rats, although it was significantly increased when the barrier was broken by aspirin or taurocholic acid under conditions of stress. The ratio of pepsin output/the net flux of H⁺ increased in stressed rats in either case of spontaneous or induced acid back-diffusion. A similar phenomenon was obtained in the 3 hr experiment (Figs. 4 and 5) using various concentrations of HCl. Thus, the action of H⁺ is probably potentiated in the gastric mucosa of rats subjected to stress. Since in our preliminary studies, the capacity of carbachol to stimulate pepsin secretion did not significantly change under conditions of stress, it is unlikely that an increase of pepsin output is due to alteration in the receptor sensitivity of chief cells. In the present study, pepsin output did not increase linearly in parallel to the amount of influxing H⁺ into the mucosa. Since pepsin secretion is regulated through biosynthesis, storage and release mechanisms, the depletion of the
storage site might account for the above phenomenon. Cheung and Chang found that the concomitant presence of ischemia (stress) and induced increase in $H^+$ back-diffusion can induce severe mucosal injury (17). This result taken together with our finding suggests that initiation or aggravation of drug-induced gastric erosions by stress might be explained by an enhancement of the action of $H^+$ in the mucosa and that there must be some mechanisms by which influxing $H^+$ is largely dissipated in the mucosa. Mucosal blood flow has been proposed to be a factor in the dissipation of $H^+$ in the gastric mucosa (8, 18, 19). In fact, an increase in mucosal blood flow is one of the consequences of acid back-diffusion caused by PCMBs, aspirin or bile salt in the removal of damaging agents from the injured portion (20). Alternatively, Davenport reported that the carbon dioxide-bicarbonate system is one of the mechanisms by which the intramucosal environment can be maintained around pH 7.4 (21). Since the distribution of carbonic anhydrase activity is found not only in the oxyntic mucosa but also equivalently in the antrum (22), $HCO_3^-$ produced by this enzyme plays an important role in neutralizing the acidic milieu and in protecting the mucosa against luminal acid in addition to the catalyzing, buffering action ($CO_2 + OH^- \rightarrow HCO_3^-$) in functionally related acid producing cells. Silen et al. demonstrated by measuring intramural pH that $HCO_3^-$ has really the capacity to neutralize acidic environment in the mucosa, where this enzyme is required to sustain a high rate of conversion of influxing $H^+$ to $CO_2$ (in the passage of $HCO_3^-$) to avoid excessive accumulation of carbonic acid and free $H^+$ in the cell (23, 24). Therefore, the amount of $H^+$ back-diffusion determined from the luminal $H^+$ loss should always be modified by such mechanisms in the mucosa, being unparallel

Fig. 7. Possible mechanism of initiation or aggravation by stress in drug-induced gastric erosions. When the gastric mucosal barrier is disrupted by barrier breakers, subsequent acid back-diffusion occurs through the damaged portion. Under the normal condition, influxing $H^+$ is largely dissipated by translocation with the blood stream or neutralization with $HCO_3^-$, while under the stress condition, the $H^+$ dissipating system is impaired, and the mucosa is exposed to considerable amounts of $H^+$, resulting in erosions. Pepsin output is one of indicators which reflect the amount of viable $H^+$ in the mucosa.
to the toxic action of H⁺ in the mucosa. Acid back-diffusion caused by taurocholic acid seemed to be largely dissipated in the mucosa as found in the case of spontaneous acid back-diffusion, but there was no appreciable damage in the gastric mucosa. However, if the H⁺ dissipating system is damaged or impaired by stress, a considerable amount of H⁺ in the mucosa would be viable and cause intramucosal acidosis or the release of several ulcerogenic substances, leading to a variety of mucosal lesions (Fig. 7). We have already found that acute systemic acidosis induced by intravenous infusion of HCl produces a similar potentiation in the intramucosal action of H⁺ as observed in conditions of stress (unpublished).

Another interesting finding is that the rate of H⁺ permeation in the spontaneous diffusion was significantly less under conditions of stress, while the mucosa had been exposed to an equal amount of H⁺ back-diffusion either in stress or normal conditions when the mucosal barrier was damaged by barrier breakers. This result led to the postulation that under physiologic conditions, the tightness of the barrier which reflects the mucosal competence containing H⁺ in the lumen, might change dynamically depending on the functional state of the stomach such as mucosal blood flow, availability of endogenous HCO₃⁻ or acid secretion, but the barrier is not a static anatomical one. However, the possibility cannot be excluded that changes in arterial pH under stress conditions, partly, contribute to the above phenomenon by lessening the chemical gradient of H⁺ between the luminal and serosal sides since spontaneous acid back-diffusion is dependent on the pH gradient between these two sides and since stress is known to induce systemic acidosis (25).

In conclusion, the development of gastric lesions requires other factors in addition to the occurrence of H⁺ back-diffusion. Stress is one of such factors which influences the dissipating system of H⁺ in the gastric mucosa. Further studies are under way to clarify the nature of this system and the relation to the mucosal barrier function and to determine which substance acts in the mucosa as a final ulcerogenic mediator.

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References


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