Body Temperature Dependency of Gastric Regional Blood Flow, Acid Secretion and Ulcer Formation in Restraint and Water-Immersion Stressed Rats

Iwao ARAI, Makoto MURAMATSU and Hironaka AIHARA
Research Center, Taisho Pharmaceutical Co., Ltd.,
1-403 Yoshino-cho, Ohmiya, Saitama 330, Japan

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Abstract—The effect of water temperature during restraint and water-immersion stress (RWIS) on gastric regional blood flow, acid secretion and ulcer formation were compared to those of restraint stress (RS) alone in rats. RS had no effect on the gastric regional blood flow. In contrast, the gastric regional blood flow was significantly decreased by RWIS. A water temperature dependent reduction of gastric regional blood flow induced by RWIS was observed between 20°C and 30°C. The decrease in gastric regional blood flow for RWIS rats was related to a lowering of the body temperature, which almost coincided with the temperature of water for the immersion. The gastric acid output was not influenced by RS. However, RWIS significantly increased the gastric acid output. The temperature of water in order of increasing acid output induced by RWIS was 25°C > 30°C > 20°C. Little ulcer formation was found in RS rats, while exposure to RWIS caused marked ulceration. The temperature of water in order of severity of ulceration by RWIS was 25°C > 20°C > 30°C. The severity of ulceration was not related to the decrease in gastric regional blood flow or increase of acid output, but was correlated to the ratio of the gastric blood flow/the acid output. These findings suggest that the decrease of gastric regional blood flow is in good agreement with the fall of body temperature, and the combined effects of the gastric blood flow and the acid secretion are involved in the ulceration caused by RWIS.

It is well known that various stressful situations, cold, noise, oscillation, restraint and external injury, cause gastric lesions in animals. Restraint and water-immersion stress (RWIS) also causes severe gastric lesions in rats or mice (1, 2). Some of the important factors related to ulceration induced by RWIS are: gastric acid secretion (3, 4), motility (5, 6), regional blood flow (7-12) and mucosal resistance (13).

Numerous studies have demonstrated a relationship between ulceration and the decrease in gastric regional blood flow (7-12). These studies suggest that spasmodic constriction of gastric blood vessels decreases the gastric regional blood flow, resulting in the development of gastric lesions.

In this study, we investigated the involvement of the gastric regional blood flow and the acid output in ulcer formation with respect to the effect of water temperature of RWIS.

Materials and Methods

Animals: Male Wistar strain rats (180–250 g) were starved for 18 hr, but allowed free access to water.

Gastric blood flow studies: Blood flow in the corpus mucosa was measured by the hydrogen gas clearance method (14). Under ether anesthesia, the animals were placed in the supine position and laparotomized. A platinum electrode (UHE-100, Unique Medical) was inserted into the gastric corpus mucosa and fixed. The animals inhaled air containing 3% hydrogen gas, regulated by a gas flowmeter (Yutaka Sangyo), for 1 min. The blood flow was measured using a hydrogen gas clearance blood flowmeter...
(PHG-201, Unique Medical) connected to a DC amplifier (AD-608G, Nihon Kohden) and recorded by an ink-writing oscilloscope (WI-681G, Nihon Kohden). Before RWIS loading, hydrogen gas was inhaled twice at 15 min intervals; thereafter, it was inhaled every 15 min for 2.5 hr during RWIS loading.

**Gastric acid secretion studies:** The pylorus was ligated, and a cannula was inserted into the forestomach. The gastric juice was collected in test tubes every hour for 6 hr by washing out the stomach with 10 ml of saline at 37°C. The total acid output of each sample was determined by titration with 0.01 N NaOH using phenolphthalein as an indicator.

**Stress ulcer studies:** Animals were placed in a stress cage and subjected to restraint stress (RS) alone or combined with immersion in a water bath (20–30°C) for 7 hr (15). At the end of the stress period, the rectal body temperature was measured using a thermistor transducer (TRH-PV, Nihon Kohden) via a temperature coupler and temperature module (AW-650H and AW-600H, Nihon Kohden). The animals were killed by a blow on the head. The stomachs were removed, filled with 1% formalin solution and placed into the same solution for 10 min. The specimens were then cut along the greater curvature and lesions in the glandular portion were measured. The ulcer index was determined by the area of each lesion in the stomach under stereoscopic microscopy.

**Statistical analysis:** The significance of differences between values were examined by Student’s t-test.

**Results**

**Effects of RS and RWIS on the gastric regional blood flow:** As shown in Fig. 1, the corpus mucosal blood flow was not affected by RS at room temperature. In contrast, the gastric regional blood flow was significantly decreased by RWIS, and reached a plateau 120 min after the water-immersion. The temperature of the water in order of decreasing gastric corpus blood flow was 20°C>25°C>30°C.

**Effects of RWIS on the gastric acid secretion:** The gastric acid output in rats was not affected by RS during the experimental periods (Fig. 2). However, RWIS in the 20–30°C water bath significantly increased the gastric acid output. These effects gradually subsided in RWIS rats. The temperature of the water in order of increasing of gastric acid output in the early phase (1–2 hr) was 25°C>30°C>20°C.

**Effect of water bath temperature on ulcer formation and body temperature in RS and**

![Fig. 1. Effects of body temperature on gastric corpus blood flow in RS and RWIS rats. RS, restraint alone stress; RWIS, restraint and water-immersion stress. °C indicates the temperature of the water bath. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the value before water-immersion (paired sample, t-test).](image1)

![Fig. 2. Effects of body temperature on gastric acid secretion in RS and RWIS rats. RS, restraint alone stress; RWIS, restraint and water-immersion stress. °C indicates the temperature of the water bath. Each value represents the mean±S.E. *P<0.05, **P<0.01 and ***P<0.001, compared with the values before water-immersion (paired sample, t-test).](image2)
RWIS rats: The body temperature in RWIS rats decreased to almost the same temperature as the water-immersion bath in 45 min after the water-immersion, and the body temperature did not change during the experimental periods. Exposure to RWIS induced marked ulceration, while little ulceration was observed in RS rats (Table 1). The most severe ulceration was induced by the RWIS at 25°C. The temperature of the water in order of severity of the ulceration was 25°C>20°C>30°C.

To clarify the relationship between the gastric blood supply and acid secretion in ulcer formation in RWIS rats, we devised an index to assess the blood supply with respect to the acid output according to the equation: gastric corpus blood flow (GCBF)/amount of acid output (AO) in the early phase (1 hr from the beginning of water-immersion). A strong correlation was observed between the GCBF/AO index and the ulcer index (Table 1).

### Discussion

This study demonstrated a relationship between the gastric blood flow and the acid output, which are important factors in the ulcer formation, under RWIS. The gastric blood flow was markedly influenced by the body temperature. The severity of ulceration caused by RWIS did not depend on the degree of the decrease in blood flow. The increase in the acid output and decrease in the blood flow were simultaneously observed under RWIS. Furthermore, the temperature of water in order of increasing activity of acid secretion was 25°C>30°C>20°C.

Numerous investigators have suggested that acid secretion or blood flow is not the only factor involved in the occurrence of ulcers under RWIS. An important factor in stress-induced ulceration appears to be gastric mucosa ischemia, which is caused by a reduction in the gastric mucosal blood flow (7). Kitagawa et al. (16) suggested that both an increase in gastric acid secretion and an insufficient blood supply were involved in the pathogenesis of gastric ulceration induced by RWIS, and the changes in the blood flow and the acid output are closely related to the ulcer formation caused by RWIS. Pare (17) suggested that the stomach lesions are a result of a disturbance in the energy metabolism of the stomach in the activity-stressed rat. In this study, at 20°C, both high energy demanded acid secretion and blood flow was smaller than those at 25°C and 30°C, while at 25°C, the acid secretion was large and almost the same or more than that at 30°C, despite of the significantly smaller blood supply compared with that at 30°C.

To investigate the degree of ischemia in the gastric mucosa with respect to the acid secretion, the relative value of the blood supply to the acid secretion may be useful.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RS N=10</th>
<th>RWIS (30°C) N=6</th>
<th>RWIS (25°C) N=6</th>
<th>RWIS (20°C) N=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (BT) (°C)</td>
<td>37.4±0.1</td>
<td>32.4±0.2</td>
<td>26.2±0.1</td>
<td>21.3±0.1</td>
</tr>
<tr>
<td>Gastric blood flow (GBF) (%)†</td>
<td>189.3±79.5</td>
<td>84.7±9.2</td>
<td>64.2±5.9**</td>
<td>37.3±5.9***</td>
</tr>
<tr>
<td>Acid output (AO) (%)†</td>
<td>97.7±11.3</td>
<td>238.7±40.5**</td>
<td>439.3±104.7***</td>
<td>160.5±25.7</td>
</tr>
<tr>
<td>GBF/AO</td>
<td>1.94</td>
<td>0.43</td>
<td>0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>Ulcer index (mm²)</td>
<td>0.03±0.02</td>
<td>0.23±0.12*</td>
<td>4.44±0.95###</td>
<td>1.23±0.33##</td>
</tr>
</tbody>
</table>

*GBF and AO were measured 1 hr after the water immersion, and ulcer index and BT were measured after 7 hr. Each value indicates the mean±S.E. †: percent of controls were calculated by comparison with the values before and after the water immersion. **P<0.01 and ***P<0.001 vs. the values before the water-immersion (paired sample, t-test). *P>0.05, **P<0.01 and ***P<0.001 vs. RS group (unpaired sample, t-test).
Thus, in this study, we used gastric corpus blood flow as a parameter of blood supply and the amount of acid output during a specified period as a parameter of acid secretion. The ulcer index in RWIS rats was closely correlated with the gastric corpus blood flow/acid output index especially during the early period of RWIS. These results suggest that the combined effects of blood flow and acid output are involved in the ulceration process caused by RWIS. Furthermore, they suggest that the body temperature plays an important role in stress-induced ulceration.

In conclusion, ulcers in RWIS rats may be induced by an insufficient mucosal blood flow, which is accompanied by an increase in acid secretion especially during the early period of RWIS and that the body temperature is involved in the severity of the ulcer in relation to the energy demand of the gastric mucosa.

References
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