Gastric Mucosal Blood Flow in Relation to Stress-Induced Hypercontraction in Spontaneously Hypertensive Rats

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Abstract The spontaneously hypertensive rat (SHR) is a widely used animal model for essential hypertension, and is less susceptible to cold restraint stress in gastric ulcer formation. We previously reported that acid secretion is low in SHR due to sympathetic facilitation compared with normotensive Wistar-Kyoto rats (WKY). The purpose of this study was to evaluate the autonomic nervous function and the gastric mucosal blood flow related to gastric motility during cold restraint stress in SHR. Male SHR and WKY, 24–28 weeks old, were used in this study. Noradrenergic innervation, noradrenaline and dopamine contents in the muscle layer were significantly greater in SHR than in WKY, and tissue choline acetyltransferase activity was significantly lower in SHR. Gastric motility was markedly enhanced by cold restraint stress in WKY. By contrast, SHR maintained the rhythmic and low amplitude contractions regardless of hypothermia. Mucosal blood flow decreased markedly during hypothermia in WKY but was well sustained in SHR. In conclusion, the increase in gastric motility associated with cold restraint stress was suppressed in SHR by sympathetic facilitation in the muscle layer, and this may have contributed to the prevention of ulcer formation by maintaining mucosal blood flow in SHR.

Key words: spontaneously hypertensive rat, gastric motility, mucosal blood flow, autonomic nervous system.

Spontaneously hypertensive rat (SHR) is a widely used animal model for essential hypertension and is associated with sympathetic hyperactivity in the peripheral organs without any treatment [1]. Several studies involving stress-induced gastric injury in SHR have been published and the results are conflicting [2–5]. Most of them supported findings that SHR is less susceptible to stress-

induced ulcers [2, 4, 5]. The mechanism of low susceptibility in SHR has not been fully understood. Pare [4] approached this problem from the standpoint of behavioral and psychological aspects since SHR are behaviorally more active than WKY. We previously reported that the susceptibility of stress-induced ulcer is inversely correlated with systemic blood pressure and that the alteration of autonomic nervous function contributes to inhibition of stress ulcers by suppressing acid secretion and motility in the hereditary hypertensive rats [6–8].

Gastric motility and mucosal blood flow are important factors for the pathogenesis of gastric mucosal injury and these functions are controlled mainly by the autonomic nervous system. The strong muscular contractions of the gastric wall produce mechanical friction between the mucosal folds and compress the intramural vessels to produce ischemia or engorgement of the mucosal capillaries [9–11], and the blocking of the cholinergic tract completely abolishes hypercontraction and mucosal injury even on the supplementation of HCl [12]. In this study, we focused on the gastric motility and mucosal blood flow during cold stress in relation to autonomic nervous system activity in SHR.

MATERIALS AND METHODS

A total of 108 male 24- to 28-week-old SHR and WKY were used in this study. SHR and WKY were purchased from Charles River Japan (Atsugi, Japan). The rats were housed in groups of three to four per cage in an air-controlled room at 24°C (lights on from 7 AM to 7 PM) at the Laboratory Animal Center of Nagasaki University. The rats were given free access to food (laboratory chow F2, Japan CLEA Co., Tokyo) and tap water *ad libitum*. Before each experiment, all food was withheld for 17 h, but water was provided.

1. *Gastric lesions and gastric motility induced by cold restraint stress at the conscious state.* Stress ulcer susceptibility was evaluated by the restraint-water immersion stress (RWI) as described by Takagi and Okabe [13]. After a 17-h fast, animals were placed in a restraint cage and immersed up to the xyphoid process for 5 h in water at 23°C. After RWI treatment, the rats were sacrificed and their stomachs were fixed with 7 ml of formalin and then opened along the greater curvature. The extent of lesions was expressed as the sum of the area (mm²) of the mucosal breaks. The strain-gauge force transducer (Star Medical Co., Tokyo) was fixed on the anterior wall of the lower corpus in parallel to the circular muscle for motility observation during RWI [12]. The motility index (MI) was calculated from the frequency and magnitude of contracted waves over a 10-min period. The formula is as follows: $MI = H \times F$ ($g \times \min^{-1}$), $H = \sum h_i/n$ (g), F : waves/min ($\min^{-1}$).

Rectal temperature was monitored with a thermistor probe (Nihon Kohden, Tokyo), 2 mm in diameter, which was inserted 5 cm into the rectum from the anus during RWI.

2. *Gastric lesions, gastric motility, and mucosal blood flow in hypothermia under urethane anesthesia.* Gastric lesion, motility, and mucosal blood flow were

measured independently under urethane anesthesia (1.0 g/kg, s.c.). The rectal temperature was controlled to be reduced from 37 to 24°C in about 90 min on the cold water bed (4–10°C) in both strains. After the rectal temperature reached 24°C, the stomach was resected and the gastric lesion index was measured as described above. Gastric motility was continuously monitored by the balloon method during the back skin cooling on the cold water bed. The recording balloon (1.1 ml), made of a thin rubber membrane and connected to a pressure transducer (Star Medical, AP-1, Tokyo), was embedded in the lower part of gastric body through a small incision of the second portion of the duodenum. For quantitative analysis of the motility, the MI was calculated from frequency and magnitude of contracted waves over a 3-min period at an arbitrary rectal temperature. The formula is as follows: $MI = H \times F$ (mmHg $\times$ min⁻¹), $H = \Sigma h_i/n$ (mmHg), F : waves/min (min⁻¹).

Gastric blood flow was determined by the hydrogen gas clearance method in the urethane anesthetized rats before cold stimulation and at a body temperature of 24°C. After a midline incision of the abdomen, the forestomach was opened and corpus mucosal blood flow was measured using platinum contact electrode and 3% hydrogen in air. One platinum contact electrode was positioned against the corpus mucosa. One reference electrode was placed inside the abdominal cavity. Three percent hydrogen in air was administered via the nasal tube until the gastric mucosa was completely saturated with hydrogen gas, as indicated by the current recorded at each platinum electrode reaching a plateau. The rate at which the current decreased after discontinuation of hydrogen administration was used to determine blood flow. In the present study, motility and mucosal blood flow between SHR and WKY were compared at the same rectal temperature because the extent of hypothermia is regarded a factor which regulates gastric motility [14].

Effects of bilateral cervical vagotomy and atropine hydrochloride pretreatment on gastric motility and gastric lesion were also examined.

3. *Tissue catecholamine content and noradrenergic histofluorescence and tissue choline acetyltransferase (CAT) activity.* For biochemical analysis and histofluorescence observation, the gastric tissues were sampled from the rats after 17 h of fasting without cold stress. The resected stomach was immediately separated into the mucosal layers and muscle layers including the serosa of the gastric corpus. Noradrenaline (NA) and dopamine (DA) contents in the body muscle layer were measured by high-performance liquid chromatography with the electrochemical detector (HPLC-ED) system, and CAT activity of the fundic muscle layer was determined by a method previously described [8]. The noradrenergic histofluorescence of Auerbach's plexus in the muscle layer was demonstrated by the glyoxalic acid fluorescence histochemistry.

4. *Data analysis.* Student's *t*-test was used to determine the statistical significance of the data, and $p < 0.05$ was regarded as significant. All data are mean $\pm$ SD.

RESULTS

1. *Gastric lesions and gastric motility induced by cold restraint stress at the conscious state*

Linear mucosal lesions were frequently encountered in the gastric body of WKY after RWI, whereas there were few in SHR, and those seen in SHR tended to be minute. Ulcer index was 9.22 ± 3.44 and 2.35 ± 0.35 mm² in WKY ($n=6$) and SHR ($n=6$) respectively ($p < 0.001$). Although there was no significant differences in the rectal temperature at resting state between the two strains, after 5 h of RWI it was 26.4 ± 0.7 and $24.9 \pm 0.3^\circ\text{C}$ in WKY and SHR, respectively ($p < 0.01$) (Fig. 1). Gastric motility, monitored with a force transducer, was greatly augmented during RWI in WKY (Fig. 2). The amplitude of contractions started to increase about 30 min after onset of RWI and reached maximal level after 3 h of stress. In contrast to WKY, enhancement of gastric motility was significantly lower in SHR. MIs during RWI at 5 h were 36.1 ± 12.6 and 8.5 ± 3.2 g/min in WKY ($n=6$) and SHR ($n=6$), respectively ($p < 0.05$).

2. *Gastric lesions gastric motility and mucosal blood flow in hypothermia under urethane anesthesia*

Gastric lesions induced by hypothermia were spotty erosions and milder in an anesthetic state in both strains than in a conscious state. The ulcer index was $1.78 \pm$

Fig. 1. Rectal temperature during restraint water immersion stress (23°C). The reduction of body temperature by restraint water immersion stress was significantly greater in SHR than in WKY.

Fig. 2. Gastric motility at restraint water immersion stress. Gastric motility was greatly enhanced by restraint water immersion and reached a maximal level in WKY, but it was slight in SHR. Arrows indicate the beginning point of stress.

Fig. 3. Gastric motility in hypothermia under urethane anesthesia. Representative data at the rectal temperatures of 37, 28, and 24°C were demonstrated. Gastric motility was enhanced gradually after cold stimulation, and especially around a rectal temperature of 28°C or below.

Fig. 4. Motility index in hypothermia under urethane anesthesia. Prolonged hypothermia induced a significant increase of motility index in WKY. In contrast, the MIs in SHR increased slightly even in hypothermia.

0.70 and $0.13 \pm 0.21 \text{ mm}^2$ in WKY ($n=6$) and SHR ($n=6$), respectively ($p < 0.001$). Gastric motility was well demonstrated by the balloon method in both strains. Generally, the amplitudes increased and frequencies decreased in accordance with the decline in body temperature. Gastric motility was remarkably enhanced by hypothermia in WKY, especially at body temperatures of 28°C or lower. Strong and irregular contractions appeared at around 24°C in WKY, however, the maximum amplitude was about half of that observed at RWI. By contrast, SHR maintained rhythmic, low-amplitude contractions regardless of hypothermia (Fig. 3). The MI increased in association with reduction of body temperature in both strains, but to different degrees (Fig. 4). In WKY, the MI showed a rapid and great increase and the maximal values to about nine times greater than the basal values. By contrast, in SHR the elevation was very slight in spite of hypothermia. The results of corpus mucosal blood flow measured by the hydrogen gas clearance method are shown in Fig. 5. There were no significant differences in gastric mucosal flow in both strains before cold stimulation (WKY, 49.5 ± 7.5 ; SHR, $48.0 \pm 10.5 \text{ ml/min/100 g}$). After cold stimulation, the extent of blood flow decline was great in WKY ($32.1 \pm 4.0 \text{ ml/min/100 g}$, $p < 0.01$), but slight in SHR ($43.0 \pm 2.3 \text{ ml/min/100 g}$).

Fig. 5. Gastric mucosal blood flow measured by the hydrogen gas clearance method before and after hypothermia. Rats were anesthetized by urethane during an experiment. At the rectal temperature of 24°C the mucosal blood flow was greatly decreased in WKY, but slight in SHR.

Enhancement of gastric motility and gastric lesions were abolished by bilateral cervical vagotomy or atropine hydrochloride pretreatment.

3. *Tissue catecholamine content, noradrenergic histofluorescence, and tissue CAT activity*

Tissue content of NA and DA were significantly greater in the muscle layer of SHR than in that of WKY (Table 1). SHR showed relative hyperinnervation of noradrenergic fibers with numerous varicosities in the intramuscle layer (Fig. 6). CAT activity was significantly lower in the muscle layer of the corpus in SHR (6.18 ± 0.34 nmol/min/g, $n = 13$, $p < 0.01$) than WKY (7.88 ± 0.34 nmol/min/g, $n = 13$). These results indicated that sympathetic innervation is well developed and that cholinergic activity is suppressed in the muscle layer of SHR.

Table 1. Tissue catecholamine content in the muscle layer.

	Noradrenaline (ng/g tissue)	Dopamine (ng/g tissue)
SHR (6)	$633 \pm 136^{**}$	$16.5 \pm 4.4^*$
WKY (6)	401 ± 96	9.7 ± 4.0

Mean $\pm$ SD. * $p < 0.05$, ** $p < 0.001$ vs WKY.

Fig. 6. Noradrenergic histofluorescence. The noradrenergic network with varicosities in the intramuscular layer was denser in SHR(A) than in WKY(B). $\times 165$.

DISCUSSION

A low susceptibility to stress-induced ulcer in SHR has been reported, however the mechanism is not fully understood. We previously reported that acid secretion, measured by the pyloric ligation method, is significantly lower in SHR by the prolonged sympathetic facilitation [6]. Although acid secretion is one of the most important causative factors, the stress-induced ulcer is a multifactorial disease. In the present study, the role of gastric motility and mucosal blood flow on stress-induced ulcer susceptibility was evaluated in SHR. We did not show the data of mucosal blood flow in a conscious state, however, because it was quite difficult for an electrode to be fixed in the submucosa due to rash body movement during RWI. Then the experiment under urethane anesthesia was carried out.

Hypothermia is an important ulcerogenic factor, as previously indicated by some investigators [14, 15]. First, Antoon and Gregg [14] demonstrated that the degree of hypothermia resulting from restraint correlates well with the severity of ulceration. Niida *et al.* [15] pointed out that prolonged hypothermia induced gastric lesions in association with acid hypersecretion and hypercontraction even under the anesthetic condition. In the present study, the reduction of body temperature during RWI was significantly greater in SHR than in WKY. However, mucosal lesions were significantly smaller in SHR than in WKY, implying that SHR has some inhibitory mechanisms against the hypothermia-induced ulcerogenesis.

There is no consensus regarding the effect of local gastric or systemic hypothermia on gastric mucosal blood flow. In a normal dog, the hypothermia caused a significant reduction in blood flow to all layers of the corpus using the radio rubidium-clearance technique [11]. A similar result was obtained by Murakami *et al.* [16] by hydrogen clearance. In contrast, in the anesthetized rat, reduction of the core temperature from 37 to 24°C by cold water immersion for up to 3 h did not produce any significant changes in gastric mucosal blood flow measured by aminopyrine clearance [17]. Garrick *et al.* [10] also indicated no significant change in overall gastric mucosal flow during cold restraint and emphasized prolonged strong contractions for a pathogenesis of mucosal damage. In SHR at a physiological state, the percent blood flow distribution to the splanchnic vascular bed is the same as in normotensive rats [18, 19]. In the present study, the decrease in gastric blood flow was significantly smaller in SHR in accordance with inhibition of gastric hypercontraction during hypothermia. By contrast, WKY showed the significant decrease in gastric blood changing reciprocally to hypercontraction during hypothermia, resulting in marked gastric lesions along the folds. These findings suggest that hypothermia-induced hypercontraction plays an important role in the reduction in mucosal blood flow in the rat stomach [12], but regulation of gastric microcirculation by adrenergic nerves during stress is complicated. The possibility of escape phenomenon from vasoconstriction [20] and post-stimulation dilatation

after noradrenergic stimulation [21] in SHR cannot be neglected as a cause of the attenuated reduction in blood flow, because SHR possesses a significantly greater level of catecholamine in the gastric mucosa than WKY [5]. Furthermore, increased blood viscosity during hypothermia should be considered a factor to decrease mucosal blood flow [22] in both strains irrespective of contractile activity.

Although many factors participate in the regulation of gastric motility and mucosal blood flow, the autonomic regulation is a major factor. Adrenergic nerves provide the stomach with a mechanism for control of acid secretion, motility, and microcirculation. Noradrenaline has been shown to regulate parasympathetic nerve-effector transmission presynaptically by reducing acetylcholine release in the digestive tract [23]. In this study, we showed a significantly higher density of adrenergic innervation and lower CAT activity in the muscle layer of SHR. In SHR, noradrenergic and dopaminergic hyperactivity may suppress gastric motility by suppressing acetylcholine release from cholinergic nerve endings through α_2 and D_2 receptors. To confirm the role of sympathetic hyperactivity in SHR, we previously demonstrated that the gastric lesions induced in SHR were aggravated by treatment with 6-hydroxydopamine, as an agent for chemical sympathectomy [8].

It is concluded that the increase in gastric motility associated with cold restraint stress is greatly inhibited in SHR by sympathetic facilitation in the muscle layer, and this might contribute to the prevention of ulcer formation with sustaining mucosal blood flow in SHR.

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