Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress

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Abstract A decrease in constitutive NO synthase (cNOS) activity and an increase in inducible NO synthase (iNOS) activity occurred with an increase in myeloperoxidase (MPO) activity, an index of neutrophil infiltration, in the gastric mucosa of rats with water immersion restraint (WIR) stress. This increase in gastric mucosal MPO activity was enhanced by pretreatment with N^G-monomethyl L-arginine, a non-selective NOS inhibitor, but was prevented with maintenance of gastric mucosal cNOS activity by pretreatment with aminoguanidine, a selective iNOS inhibitor. The MPO activity was negatively correlated with the cNOS activity in all WIR-stressed rats used ($r = -0.723$). These results suggest that a decrease in cNOS activity could contribute to an increase in neutrophil infiltration in the gastric mucosa of WIR-stressed rats.

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Key words: Water immersion restraint stress; Gastric mucosal injury; Constitutive NO synthase; Inducible NO synthase; Myeloperoxidase; Neutrophil infiltration

1. Introduction

It has been suggested that neutrophil infiltration into gastric mucosal tissues is a critical process in the pathogenesis of a variety of gastric ulcers [1–7]. Our previous study has demonstrated that neutrophil infiltration into gastric mucosal tissues is involved in the development of acute gastric mucosal lesions in rats with water immersion restraint (WIR) stress [8].

Nitric oxide (NO) is a biologically active substance which is produced from L-arginine via a Ca^{2+}-dependent constitutive NO synthase (cNOS) or a Ca^{2+}-independent inducible NO synthase (iNOS) [9]. Both NO synthases have been detected in gastric mucosal tissues of rats histochemically [10] and biochemically [11–13]. It has been widely accepted that in the digestive system, NO produced by cNOS is cytoprotective, while excessive NO produced by iNOS is cytotoxic [14]. Recently, we have reported that in rats with WIR stress, a decrease in cNOS activity and an increase in iNOS activity in the gastric mucosa occurs with the development of gastric mucosal lesions [13]. These changes in gastric mucosal cNOS and iNOS activities with the development of gastric mucosal lesions in rats with WIR stress seem to be consistent with the above-mentioned proposal.

It has been demonstrated that NOS inhibitors such as N^G-monomethyl L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) promote leukocyte-endothelial cell adhesive interactions and leukocyte emigration in postcapillary venules [15]. It has also been shown that L-NAME-induced leukocyte adhesion in postcapillary venules is inhibited by L-arginine, a precursor for NO [15], or nitroprusside, an NO donor [16]. These findings have suggested that NO should play an inhibitory role in neutrophil infiltration into tissues.

The above-mentioned findings may allow us to assume that a change in NO production in the gastric mucosa of rats with WIR stress affects neutrophil infiltration into the gastric mucosa, resulting in the development of gastric mucosal lesions. However, it is unclear at present whether or not changes in gastric mucosal cNOS and iNOS activities contribute to neutrophil infiltration into the gastric mucosa of rats with WIR stress. Therefore, we examined the relationship between the changes of cNOS and iNOS activities and the change of myeloperoxidase (MPO) activity, a marker of neutrophil infiltration [17], in the gastric mucosa of WIR-stressed rats with and without pretreatment of either L-NMMA, which is known to inhibit both cNOS and iNOS [18], or aminoguanidine, a selective inhibitor of iNOS [9,19].

2. Materials and methods

2.1. Chemicals

Tetramethylbenzidine, oxyhaemoglobin and aminoguanidine were purchased from Sigma Chemical Co. (St. Louis, MO, USA); rabbit anti-rat polymorphonuclear (PMN) antiserum from Inter-Cell Technologies, Inc. (Hopewell, NJ, USA); L-arginine, L-valine, N^G-monomethyl L-arginine monoacetate (L-NMMA), N^G-monomethyl D-arginine monoacetate (D-NMMA), and other chemicals from Wako Pure Chemicals Ind., Ltd. (Osaka, Japan).

2.2. Induction of gastric mucosal lesions

Seven-week-old male Wistar rats weighing 200–210 g purchased from Shizuoka Laboratory Animal Center Co. (Hamamatsu, Japan), were used. These animals were starved for 24 h prior to experiments, but were allowed free access to water. Rats were restrained in a wire cage and immersed up to the depth of the xiphoid process in a 23°C water bath for 1, 3 and 6 h to produce WIR stress-induced gastric mucosal lesions as described by Takagi and Okabe [20]. Rats were killed under ether anesthesia after application of 0, 1, 3, and 6 h of WIR at which time stomachs were removed. The removed stomachs were cut open along with the greater curvature and the gastric mucosa was removed on ice using small scissors. For the observation of gastric lesion, the stomachs of rats with and without WIR, after ligation of the esophagus at 5 mm proximal to the gastroesophageal junction and the duodenum at 10 mm distal to the pylorus, were infused with approximately 10 ml of saline (50 ml/kg body weight) and secured by ligatures at both esophagus and duodenum, and then the stomachs were removed. The removed stomachs were fixed with 10% formaldehyde for 10 min and cut open along with the greater curvature. The gastric mucosa was carefully examined, independently, by two observers in a blind manner, for lesions recognized as linear breaks (erosions) at the mucosal surface of the glandular part under a stereo-

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scopic microscope (×10). The extent of the lesion (lesion index) is expressed as the sum of the length of these breaks per stomach. All animals received human care in compliance with the guideline of the Animal Care and Use Committee of Fujita Health University.

2.3. Assays of gastric mucosal MPO

Immediately after gastric mucosal collection, the mucosa was disrupted using a microhomogenizer in 9 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5), and the prepared homogenate was sonicated on ice for 60 s using a Handy Sonic model UR-20P (Tomy Seiko Co., Tokyo). The sonicated homogenate was centrifuged at $10\,000 \times g$ for 20 min at 4°C, and the resultant supernatant was used for the assay sample. Gastric mucosal MPO was assayed by the method of Suzuki et al. [21] measuring the hydrogen peroxide-dependent oxidation of tetraethylbenzidine at 37°C. One unit (U) of this activity is defined as the amount of enzyme causing a change in absorbance of 1.0 per min at 655 nm.

2.4. Measurement of NOS activity

Gastric mucosal NOS activity was measured spectrophotometrically using the oxidation of oxyhaemoglobin to methaemoglobin by NO as described previously [22,23]. The absorption difference between 401 and 421 nm was continuously monitored with a dual wavelength recording spectrophotometer (Hitachi UV-2000) at 37°C. For total NOS assay, the incubation medium contained 1.6 µM oxyhaemoglobin, 200 µM $CaCl_2$, 1 mM $MgCl_2$, 100 µM L-arginine, 100 µM NADPH, 40 mM potassium phosphate pH 7.2, 1 mM N^G-nitro-L-arginine, and 10% v/v tissue extract with 50 mM L-valine to inhibit arginase [23]. For cNOS assay, 1 mM EGTA was added to the above incubation medium without N^G-nitro-L-arginine. Oxyhaemoglobin oxidation was confirmed as being caused by NO synthesis. iNOS activity was calculated by subtraction of cNOS activity from total NOS activity.

2.5. Drug treatment

Rats were injected subcutaneously with L-NMMA (100 mg/kg), its D-enantiomer, D-NMMA (100 mg/kg) or aminoguanidine (100 mg/kg), which was dissolved in isotonic saline, at 0.5 h prior to WIR stress. Control rats were injected subcutaneously with the same volume of isotonic saline. To determine the specificity of L-NMMA, L-arginine (300 mg/kg) or D-arginine (300 mg/kg) was injected intraperitoneally 15 min before L-NMMA administration. Rabbit anti-PMN antiserum (10 ml/kg), which shows agglutination titer against purified rat PMN at 1:256 dilution, was intraperitoneally injected 18 h before the beginning of the WIR stress. Serum obtained from normal rabbit was given to control rats in the same manner.

2.6. Statistical analysis

Results obtained are expressed as means ± S.D. Results are analyzed by computerized statistical packages (SuperANOVA, Statview II) if necessary. Each mean value is compared by one-way analysis of variance and Fisher's protected least significant difference for multiple comparisons as the post hoc test. The level of significance was taken as $P < 0.05$.

3. Results

Gastric mucosal lesions developed in rats subjected to WIR stress over a 6 h period and the magnitude and development of gastric mucosal lesions in the present study were identical with those shown in our previous reports [8,13] (Fig. 1A). In contrast, there were no gastric mucosal lesions in rats without WIR stress (data not shown). MPO activity, an indicator of neutrophil infiltration [17], in the gastric mucosa of rats subjected to WIR stress for 3 and 6 h significantly increased (Fig. 1A). Gastric mucosal cNOS activity significantly decreased in rats subjected to WIR stress for 3 and 6 h (Fig. 1B). In contrast, iNOS activity in the gastric mucosa of rats with WIR stress for 1, 3, and 6 h significantly increased in a time-dependent manner (Fig. 1B). However, cNOS and iNOS activ-
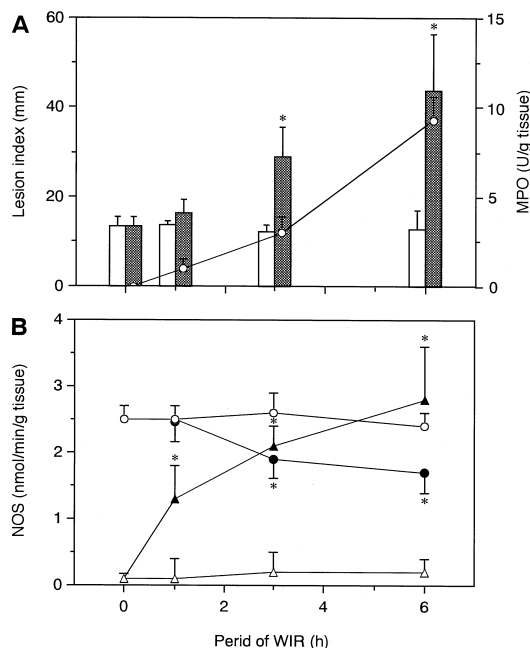

Fig. 1. Gastric mucosal lesion development and change in MPO activity (A), and changes in cNOS and iNOS activities (B) in gastric mucosal tissues of rats with and without WIR stress over a 6 h period. A: Gastric mucosal lesion index (open circle) of rats with WIR stress, and MPO activity in gastric mucosal tissues of rats with (shaded bars) and without (open bars) WIR stress were determined as described in Section 2. B: Assays of cNOS in gastric mucosal tissues of rats with (closed circle) and without (open circle) WIR stress and of iNOS in gastric mucosal tissues of rats with (closed triangle) and without (open triangle) WIR stress were performed as described in Section 2. Each value represents the mean ± S.D. for 5–11 animals. *$P < 0.05$ compared with rats without WIR stress.

ities in the gastric mucosa of control rats without WIR stress did not change during the experimental period (Fig. 1B).

When L-NMMA (100 mg/kg) with and without either L-arginine (300 mg/kg) or D-arginine (300 mg/kg), D-NMMA (100 mg/kg) or aminoguanidine (100 mg/kg) was pretreated to rats with 3 h of WIR stress, gastric mucosal lesion development and gastric mucosal MPO, cNOS, and iNOS activities were changed as shown in Table 1. Pretreatment with L-NMMA significantly enhanced the development of gastric mucosal lesion in rats subjected to WIR stress for 3 h and the level of the enhanced gastric mucosal lesions was 181% of that of the vehicle-treated group, while pretreatment with the enantiomer, D-NMMA did not augment the lesion development. In addition, the enhancement of the development of gastric mucosal lesion in L-NMMA-pretreated rats with WIR stress was reversed by exogenous L-arginine, which was administered 15 min prior to L-NMMA treatment, but not D-arginine. Pretreatment with aminoguanidine significantly attenuated gastric mucosal lesion development in rats with 3 h of WIR stress and the level of the attenuated gastric mucosal lesions was 66% of that of the vehicle-treated group. Rats without WIR stress showed no gastric mucosal lesions at 3 h after treatment with either L-NMMA or aminoguanidine.

Pretreatment with L-NMMA, but not D-NMMA, significantly enhanced the increase in MPO activity in the gastric mucosa of rats with 3 h of WIR stress, although this L-NMMA treatment did not alter gastric mucosal MPO activity
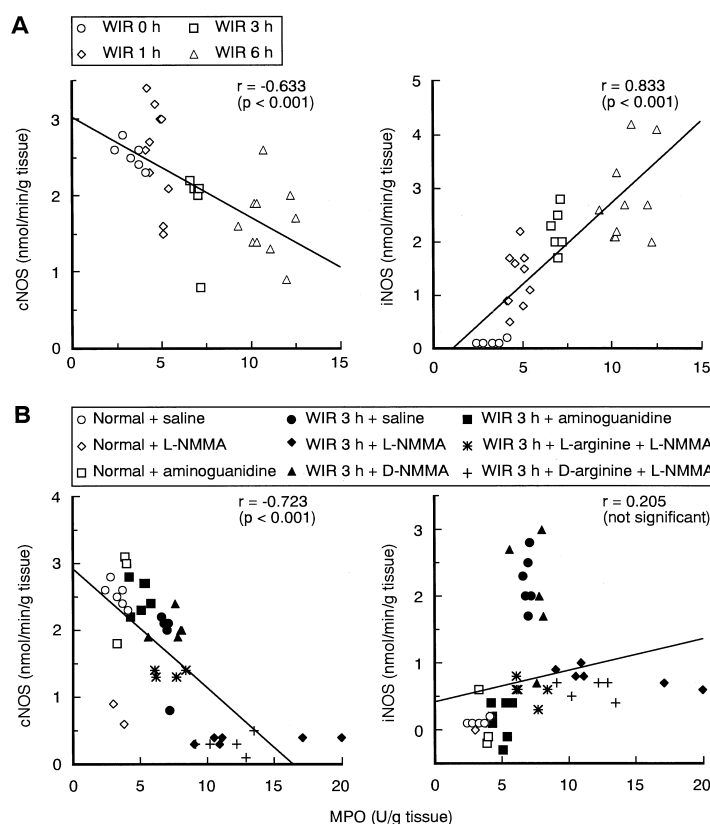
Fig. 2. Correlation between either cNOS or iNOS activity and MPO activity in gastric mucosal tissues of WIR-stressed rats without any pretreatment (A) and of all WIR-stressed rats with and without pretreatments with NO synthase inhibitors.

in control rats without WIR stress. The enhancement of gastric mucosal MPO activity in WIR-stressed rats with L-NMMA pretreatment was reversed by exogenous L-arginine, but not D-arginine. In contrast, an increase in MPO activity in the gastric mucosa of rats with 3 h of WIR stress was significantly attenuated by pretreatment with aminoguanidine, although this aminoguanidine treatment did not affect gastric mucosal MPO activity in control rats without WIR stress.

Furthermore, gastric mucosal cNOS activity in control rats without WIR stress was significantly reduced 3 h after pre-

treatment with L-NMMA, but not aminoguanidine. Neither L-NMMA nor aminoguanidine treatment had any effect on gastric mucosal iNOS activity in the control rats. An increase in gastric mucosal iNOS activity in vehicle-treated rats with WIR stress was dramatically attenuated by pretreatment with L-NMMA, but not D-NMMA, although the decreased iNOS activity in the gastric mucosa of L-NMMA-pretreated rats was still significantly higher than that in normal rats without WIR stress. A decrease in gastric mucosal cNOS activity in vehicle-treated rats with WIR stress was further enhanced by

Table 1
Effect of pretreatment of either L-NMMA or aminoguanidine on gastric mucosal lesion development and changes in gastric mucosal MPO, cNOS, and iNOS activities in rats with WIR stress

Animals	Lesion index (mm)	MPO (U/g tissue)	cNOS (nmol/min/g tissue)	iNOS (nmol/min/g tissue)
Normal control				
+Vehicle	0	3.3 ± 0.6	2.5 ± 0.2	0.1 ± 0.1
+L-NMMA	0	3.4 ± 0.5	0.6 ± 0.3	0.1 ± 0.1
+Aminoguanidine	0	3.7 ± 0.4	2.6 ± 0.7	0.1 ± 0.4
WIR (3 h)				
+Vehicle	12.0 ± 3.0	7.1 ± 0.8[a]	1.9 ± 0.5[a]	2.1 ± 0.3[a]
+L-NMMA	21.7 ± 7.2[b]	12.5 ± 4.3[a,b]	0.4 ± 0.1[a,b]	0.8 ± 0.2[a,b]
+D-NMMA	11.2 ± 3.7	7.4 ± 1.0[a]	2.0 ± 0.2[a]	2.0 ± 0.9[a]
+L-NMMA+L-arginine	12.2 ± 3.5	6.9 ± 1.1[a]	1.3 ± 0.1[a,b]	0.6 ± 0.2[a,b]
+L-NMMA+D-arginine	22.2 ± 1.9[b]	11.6 ± 1.9[a,b]	0.3 ± 0.2[a,b]	0.6 ± 0.2[a,b]
+Aminoguanidine	7.9 ± 1.9[b]	4.9 ± 0.7[a,b]	2.5 ± 0.2[b]	0.2 ± 0.3[b]

L-NMMA (100 mg/kg), D-NMMA (100 mg/kg) or aminoguanidine (100 mg/kg) was subcutaneously administered to rats 0.5 h before the onset of WIR stress. The same volume of physiological saline used as a vehicle was given to control rats in the same manner. L-arginine (300 mg/kg) or D-arginine (300 mg/kg) was intraperitoneally administered to rats 45 min before the onset of WIR stress. Each value represents the mean ± S.D. for 5–11 animals.
[a] $P < 0.05$ compared with vehicle-treated rats without WIR stress.
[b] $P < 0.05$ compared with vehicle-treated rats with WIR stress.

Table 2
Effect of pretreatment of anti-PMN antiserum on gastric mucosal lesion development and changes in gastric mucosal MPO, cNOS and iNOS activities in rats with WIR stress

Animals	Lesion index (mm)	MPO (U/g tissue)	cNOS (nmol/min/g tissue)	iNOS (nmol/min/g tissue)
Normal control	0	3.2 ± 0.3	2.6 ± 0.3	0.1 ± 0.1
WIR (3 h)				
+Normal rabbit serum	12.0 ± 2.5	7.2 ± 1.6^a	2.0 ± 0.2^a	2.1 ± 0.3^a
+Anti-PMN antiserum	7.8 ± 2.7^b	4.3 ± 0.5^b	2.3 ± 0.1^a	0.4 ± 0.4^b

Rats received i.p. administration of anti-polymorphonuclear leukocyte (PMN) antibody (10 ml/kg) 18 h prior to the onset of WIR stress. Rats without anti-PMN pretreatment received i.p. administration of an equal volume of normal rabbit serum. These rats were subjected to WIR stress for 3 h. Each value represents the mean $\pm$ S.D. for 7–10 animals.
[a] $P < 0.05$ compared with normal control rats without WIR stress.
[b] $P < 0.05$ compared with WIR-stressed rats with normal rabbit serum pretreatment.

pretreatment with L-NMMA, but not D-NMMA. The decrease of cNOS activity found in the gastric mucosa of L-NMMA-pre-administered rats with WIR stress was attenuated by co-pretreatment with L-arginine ($P < 0.05$). However, the decrease of gastric mucosal iNOS activity in L-NMMA-pretreated rats with WIR stress was not changed by co-pretreatment with L-arginine and L-NMMA. Co-treatment with D-arginine and L-NMMA had no effect on the changes of cNOS and iNOS activities in the gastric mucosa of L-NMMA-pretreated rats with WIR stress. On the other hand, an increase in iNOS activity in the gastric mucosa of vehicle-treated rats with WIR stress was significantly inhibited with maintenance of cNOS activity near the normal level by aminoguanidine pretreatment.

The correlation coefficient between gastric mucosal MPO activity and either gastric mucosal cNOS or iNOS activity was -0.633 ($P < 0.001$) or 0.833 ($P < 0.001$), respectively, in rats subjected to WIR stress alone for over a 6 h period (Fig. 2A). When rats received NOS inhibitors and their related compounds prior to WIR stress, the correlation coefficient between gastric mucosal MPO activity and either gastric mucosal cNOS or iNOS activity was -0.723 ($P < 0.001$) or 0.205 (not significant), respectively.

As shown in Table 2, anti-PMN antiserum pretreatment prevented the development of gastric mucosal lesions in rats with 3 h of WIR stress. The activity of gastric mucosal MPO in anti-PMN antiserum-pretreated rats subjected to 3 h of WIR stress was significantly lower than that in rats with normal rabbit serum pretreatment. Gastric mucosal cNOS activity in rats subjected to WIR stress for 3 h was not affected by anti-PMN antiserum pretreatment. In contrast, iNOS activity in the gastric mucosa of anti-PMN antiserum-pretreated rats with 3 h of WIR stress was dramatically attenuated compared with that of normal rabbit serum-pretreated rats with the same period of WIR stress.

4. Discussion

In this study, we examined whether or not changes in gastric mucosal cNOS and iNOS activities contribute to neutrophil infiltration into the gastric mucosa of rats with WIR stress. The neutrophil infiltration into the gastric mucosal tissues was checked by MPO activity, because MPO assay has been widespread used as an index of neutrophil infiltration in various experimental gastric injuries [24–31], colitis [32], and human gastric ulcer [33]. The development of gastric mucosal lesions was found to occur with an increase in gastric mucosal MPO activity in rats subjected to WIR stress over a 6 h period. Gastric mucosal cNOS activity significantly de-

creased in rats with 3 and 6 h of WIR stress. In contrast, iNOS activity in the gastric mucosa of rats subjected to WIR stress for 1, 3, and 6 h significantly increased and this increase in activity occurred time-dependently.

NOS inhibitors such as L-NMMA and L-NAME have been shown to promote leukocyte-endothelial cell adhesive interactions and leukocyte emigration in postcapillary venules [15]. This NOS inhibitor-induced leukocyte adhesion has also been shown to be attenuated by L-arginine, a precursor for NO [15] or nitroprusside, an NO donor [16]. These findings prompted us to examine the changes of gastric mucosal cNOS and iNOS activities and of neutrophil infiltration into gastric mucosal tissues following the development of gastric mucosal lesions using WIR-stressed rats pretreated with NOS inhibitors. As reported previously [13], the treatment of L-NMMA, a non-selective NOS inhibitor [18], but not the enantiomer D-NMMA, prior to the initiation of WIR stress significantly enhanced the development of gastric mucosal lesions with inhibition of gastric cNOS and iNOS activities in rats with 3 h of WIR stress, although the inhibited gastric mucosal iNOS activity was above the normal level. This pretreatment of L-NMMA, but not D-NMMA was found to enhance an increase in MPO activity in the gastric mucosa of rats with 3 h of WIR stress, although this L-NMMA pretreatment had no effect on the basal gastric mucosal MPO activity in control rats without WIR stress. Furthermore, the enhancements of gastric mucosal lesion development and the increase of gastric mucosal MPO activity in the L-NMMA-pretreated rats with WIR stress were completely prevented with an incomplete recovery of inhibited cNOS activity, but not inhibited iNOS activity, in the gastric mucosa by co-treatment with L-arginine, but not D-arginine. These results suggest that the enhancement of an increase in neutrophil infiltration associated with lesion development in the gastric mucosa of L-NMMA-pretreated rats with WIR stress could be mainly due to an excessive inhibition of gastric mucosal cNOS activity.

When rats pretreated with aminoguanidine, a selective iNOS inhibitor [9,19], were subjected to WIR stress for 3 h, the development of gastric mucosal lesions was incompletely prevented with a complete recovery of decreased cNOS activity and a complete inhibition of increased iNOS activity in the gastric mucosa as reported previously [13]. This pretreatment of aminoguanidine was found to cause an incomplete inhibition of increased gastric mucosal MPO activity in the WIR-stressed rats, although this aminoguanidine pretreatment did not alter gastric mucosal MPO activity in control rats without WIR stress. These results suggest that an increase in neutrophil infiltration associated with lesion development in the gastric mucosa of WIR-stressed rats could be, at least in part,

related to a decrease in gastric mucosal cNOS activity and/or an increase in gastric mucosal iNOS activity.

We further attempted to check the correlation between MPO activity and either cNOS or iNOS activity in the gastric mucosa of WIR-stressed rats with and without pretreatment of either L-NMMA (with and without L-arginine or D-arginine), D-NMMA or aminoguanidine. A relatively good negative correlation between MPO activity and cNOS activity was found in the gastric mucosa of all WIR-stressed rats with and without each pretreatment. This finding suggests that in rats with WIR stress, a decrease in gastric mucosal cNOS activity could facilitate neutrophil infiltration into the gastric mucosa, resulting in the development of gastric mucosal lesions. Accordingly, it seems that maintenance of cNOS activity in gastric mucosal tissues contributes to the prevention of neutrophil infiltration into the tissue. In addition, a definite positive correlation between MPO activity and iNOS activity was observed in the gastric mucosa of WIR-stressed rats without any pretreatment. From this finding, one can assume that an increase in iNOS activity in gastric mucosal tissues contributes to an increase in neutrophil infiltration into the tissue. However, no correlation between MPO activity and iNOS activity was found in the gastric mucosa of all WIR-stressed rats with and without each pretreatment, suggesting no contribution of increased gastric mucosal iNOS to the increase of neutrophil infiltration into the gastric mucosa of rats with WIR stress.

As afore-mentioned, gastric mucosal iNOS and MPO activities showed a similar increase during the development of gastric mucosal lesions in rats with WIR stress. The source of increased iNOS in the gastric mucosa of rats with WIR stress is still unclear at present. But, it has been reported that iNOS is induced in activated neutrophils [34,35]. From these findings, it seems likely that a part of the increase of iNOS activity found in the gastric mucosa of rats with WIR stress is due to activated neutrophils infiltrated into the tissue. Therefore, we attempted to clarify whether or not neutrophil infiltration into the gastric mucosa of rats with WIR stress contributes to an increase in iNOS activity in the tissue and this contribution is associated with the development of gastric mucosal lesions. Namely, we examined the effect of pretreatment of anti-PMN antiserum on the development of gastric mucosal lesions and the changes of gastric mucosal MPO, cNOS, and iNOS activities in rats subjected to WIR stress for 3 h. Pretreatment with anti-PMN antiserum attenuated the development of gastric mucosal lesions with prevention of an increase in gastric mucosal MPO activity in the WIR-stressed rats as shown in our previous report [8]. In addition, this pretreatment of anti-PMN antiserum prevented an increase in gastric mucosal iNOS activity without affecting a decrease in gastric mucosal cNOS activity in the WIR-stressed rats. These results indicate that neutrophil infiltration into the gastric mucosa of rats with WIR stress contributes to an increase in iNOS activity in the tissue, resulting in the development of gastric mucosal lesions, and suggest that activated neutrophils infiltrated into the gastric mucosa of WIR-stressed rats could be one of the sources of iNOS increasing in the tissue. However, a significant increase in iNOS activity in the gastric mucosa of rats subjected to 1 h of WIR stress was found without a significant increase in MPO activity in the gastric mucosal tissue at this time point. Therefore, another source of iNOS induction except neutrophils should be asso-ciated with the increase in iNOS activity found in the gastric mucosa of rats with 1 h of WIR stress.

Gastric peroxidase has been purified and characterized [36], and this enzyme exists in rats gastric parietal cells and the other mucosal cells [37]. It has been reported that in rats with cold restraint stress which is essentially the same as WIR stress, gastric peroxidase activity time-dependently decreases through the oxygen free radical-mediated inactivation of the enzyme [38,39]. We have reported that generation of oxygen free radical is closely related to the development of gastric mucosal lesions in rats with WIR stress [8]. Accordingly, it can be thought that a decrease in gastric peroxidase activity should occur in rats with WIR stress. In this study, however, gastric mucosal MPO activity increased with the development of gastric mucosal lesions in rats with WIR stress, and the increase in activity was attenuated when rats with anti-PMN antiserum pretreatment were subjected to WIR stress. The findings may allow us to think that increased MPO activity in the gastric mucosa of rats with WIR stress corresponds to neutrophil-related peroxidase activity.

In conclusion, the results obtained in this study indicate that in rats with WIR stress, a decrease in cNOS activity, but not an increase in iNOS activity, in the gastric mucosa contributes to an increase in neutrophil infiltration into the tissue, resulting in the development of gastric mucosal lesions and that neutrophil infiltration into the gastric mucosa of WIR-stressed rats contributes to an increase in iNOS activity in the tissue. The present results also suggest that NO synthesized by gastric mucosal cNOS could play an inhibitory role in neutrophil infiltration into gastric mucosal tissues and that activated neutrophils infiltrated into gastric mucosal tissues could be one of the sources of iNOS increasing in the tissue.

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