Nitric Oxide as a Factor of Genetically Determined Resistance to Stress Damages and Adaptive Protection

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August rats are more resistant to stress-induced gastric damages than Wistar rats. These interstrain differences were abolished after blockade of nitric oxide (NO) synthesis with NO-synthase inhibitor L-NNA, which indicates that NO contributes to genetically determined resistance to stress-induced injuries. Repeated treatment with L-NNA caused gastric ulceration in Wistar, but not in August rats. This is probably related to higher basal production and more intensive accumulation of NO in August rats compared to Wistar rats. Administration of L-NNA during adaptation to hypoxia suppressed its protective effects on the stomach in stress, which indicates that NO acts as the factor of adaptive protection.

Key Words: *nitric oxide; August rats; Wistar rats; stress; adaptation*

Previous studies showed that August rats are more resistant to gastric ulceration and behavioral disorders caused by acute stress compared to Wistar rats. Adaptation to hypoxia (AH) prevents stress-induced injuries [4]. NO content in August rats under normal and stress conditions is higher than in Wistar rats [3,5]. AH intensifies NO production in the organism [2,5,13]. Stress-induced damages to the stomach are mediated by ischemic adrenergic mechanisms [8]. NO blocks the release of norepinephrine from sympathetic nerve terminals and, therefore, inhibits adrenergic effects [7,12] and vasoconstriction [6]. Blockade of NO synthesis potentiates, while NO donors prevent gastric ulceration during stress [9,11]. These data suggest that the resistance of August rats to stress-induced gastric damages is related to genetically determined intensive NO synthesis. The protective effect of AH is probably associated with activation of NO production. Here we compared the effects of NO synthesis blockade by the NO synthase inhibitor L-NNA on the severity of acute stress-induced gastric ulcers in August and Wistar rats adapted and not adapted to hypoxia.

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MATERIALS AND METHODS

Experiments were performed on male August and Wistar rats weighing 177±10 and 264±11 g, respectively. To produce stress the rats were placed into a standard cage filled with water (21°C) and covered with a grid for 30 min [1]. The animals were adapted to hypoxia in a hypobaric pressure chamber of the extract-and-influx type. AH was performed at a simulated altitude of 5000 m above sea level for 10, 20, and 30 min on days 1, 2, and 3, respectively; the course was repeated after 2 days (a total of 6 procedures) [4]. The animals were stressed 1 day after completion of AH. These rats were decapitated 2 h after stress. L-NNA (Biomedicals Inc. ICN) was injected intraperitoneally in a daily dose of 20 mg/kg to adapted (1 h before AH) and unadapted rats for 6 days. Unadapted animals received the last injection of L-NNA 1 h before stress. Nonstressed rats receiving L-NNA were killed 1 day after the last injection of this preparation. Placebo group animals not receiving L-NNA were injected with 0.2 ml physiological saline (similarly to L-NNA). The total length and area of gastric ulcers were measured. We performed 8 series on August and Wistar rats: stress+placebo, AH+placebo, adaptation+stress+placebo; L-NNA; L-NNA+stress; L-NNA+adaptation; L-NNA+

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adaptation+stress; and control (intact placebo animals). The results were analyzed by Student's *t* test.

RESULTS

Acute stress caused gastric damages in unadapted Wistar rats and, to a lesser extent, in August rats (Table 1), which is consistent with published data [4,5]. Treatment with L-NNA induced gastric ulceration in intact Wistar, but not in August rats. The severity of gastric ulcers in intact Wistar rats receiving L-NNA was lower than in stressed animals (Table 1). Thus, August rats were more resistant to the influence of pharmacological NO deficiency on the gastric mucosa than Wistar rats. Previous studies showed that NO deficiency plays an important role in gastric ulceration [10]. Therefore, these interstrain differences are probably related to higher basal production [3,5] and more intensive accumulation of NO in August rats compared to Wistar rats [5].

L-NNA potentiated stress-induced gastric damages in August and Wistar rats (Table 1), which is consistent with published data [9,11]. The mean area of gastric ulcers in stressed Wistar rats treated with L-NNA 5-fold surpassed that in animals receiving placebo (Table 1). In stressed August rats receiving L-NNA the mean area of gastric ulcers was also higher than in the placebo group. It should be emphasized that these animals had not only linear, but also punctate gastric damages (as differentiated from the placebo group, Table 1). Our results indicate that the genetically determined resistance to stress-induced gastric injuries depends on NO production, which is also genetically determined.

AH prevented the development of stress-induced gastric damages in Wistar rats, which is consistent with published data [4,5]. The incidence of gastric ulcers in adapted Wistar rats was 2 times lower than in unadapted animals. The mean number of stress-induced linear ulcers, their area, and total area of ulcers in adapted Wistar rats were lower than in unadapted animals by 5 and 18 times, respectively (Table 1). AH produced no adaptive effects in August rats. Treatment of Wistar rats with L-NNA during AH caused gastric ulceration. The severity of gastric ulcers in these rats was much lower than in unadapted animals receiving L-NNA (Table 1). The mean number and area of linear ulcers in adapted Wistar rats receiving L-NNA were lower than in unadapted animals by 6 and 3 times, respectively (Table 1). Thus, AH prevented the development of stress ulcers and abolished the ulcerogenic effect of L-NNA in Wistar rats. AH and treatment with L-NNA did not induce ulceration in August rats. However, we found small gastric ulcers in August rats receiving L-NNA during AH (Table 1). The mechanisms of these changes require further investigations.

TABLE 1. Effects of NO Synthesis Inhibitor on Stress-Induced Gastric Damages in August (Numerator) and Wistar (Denominator) Rats Adapted and Not Adapted to Hypoxia ($M \pm m$, n=5-7)

Parameter	Stress+ placebo	Adaptation+ stress	L-NNA			
			intact	+stress	+adaptation	+adaptation +stress
Number of rats with ulcers	1 / 5	6 / 3	0 / 5	6 / 5	2 / 4	6 / 6
Mean number of ulcers						
linear	0.2±0.2 / 6.6±2.2*	0 / 1.0±0.2*+	0 / 12.0±3.2*	6.0±2.3+ / 9.0±2.4+	1.00±0.16o / 2.2±0.6*o	3.5±0.8x / 5.20±1.16x
punctate	0 / 0	1.5±0.3+ / 1.4±1.0+	0 / 1.0±1.0*	4.16±3.6+ / 0	2.0±1.8o / 2.0±1.0o	10.3±1.6x / 1.00±0.33*
Ulcer area, mm²						
linear	0.06±0.06 / 6.61±2.12*	0 / 0.43±0.05*+	0 / 4.38±0.82*	3.01±0.29+ / 33.9±9.7*+	0.05±0.02o / 1.90±0.11*o	0.9±0.2x / 7.0±0.8*x
punctate	0 / 0	0.063±0.020+ / 0.01±0.01*	0 / 0.1±0.1*	0.15±0.15+ / 0	0.02±0.01o / 0.02±0.01o	0.38±0.18x / 0.010±0.003*
total	0.06±0.06 / 6.61±2.12*	0.063±0.020 / 0.44±0.23*+	0 / 4.4±0.8*	3.17±0.44+ / 33.9±9.7*+	0.07±0.06o / 1.93±0.80*o	1.34±0.23x / 7.0±1.0*x

Note. Test parameters in control and adapted August and Wistar rats are equal to zero. Significant differences compared to *August rats and +stress+placebo, oadaptation, and xadaptation+stress groups.

Blockade of NO synthesis during AH abolished its protective effects in stressed Wistar rats. After stress, test parameters in adapted animals receiving L-NNA practically did not differ from those in unadapted rats (Table 1). However, in Wistar rats receiving L-NNA, AH suppressed the potentiating effect of this preparation on the development of stress damages. In rats treated with L-NNA during AH the mean number and area of stress ulcers were lower than in unadapted animals by 1.9 and 4 times, respectively (Table 1).

AH did not prevent the development of stress-induced gastric ulcers in August rats receiving L-NNA (Table 1). By contrast, the ulcerogenic effect of AH was more pronounced in stressed animals after blockade of NO synthesis. Stress produced severe gastric ulcers in all adapted August rats treated with L-NNA, but led to insignificant ulceration only in 1 unadapted animal receiving placebo (Table 1).

It should be emphasized that treatment with physiological saline (placebo) did not cause gastric ulceration.

Our results indicate that the genetically determined resistance to stress-induced gastric damages depends on NO production, which is also genetically determined. August rats are more resistant not only to the development of stress-induced gastric ulcers, but also to ulceration associated with pharmacological NO deficiency (compared to Wistar rats). We revealed considerable interstrain differences in the protective effect of AH during acute stress and pharmacological NO deficiency. Probably, AH is followed by a more significant increase in NO content in adapted Wistar rats than in August rats. During AH the intensity of NO accumulation in the vascular wall in August rats is higher than in Wistar rats. Moreover, this process probably dominates over NO production in adapted animals [5]. These data show that NO is a key factor of the genetically determined resistance to acute stress-induced damages and adaptive protection.

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