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Nitric oxide inhibition intensifies cold-restraint induced gastric ulcers in rats

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Abstract. Treatment 20 min beforehand with an inhibitor of nitric oxide (NO) synthesis, N^W-nitro-l-arginine methyl ester (L-NAME) (12.5, 25, 50 or 100 mg/kg, s.c.), dose-dependently intensified gastric glandular mucosal ulceration produced by cold-restraint stress. Hexamethonium (20 mg/kg) or atropine (1 mg/kg) pretreatment s.c. 20 min before stress strongly antagonised stress-evoked ulceration, as well as the ulcer-potentiating effects of L-NAME when either cholinoceptor antagonist was given concurrently with the NO inhibitor. Stress-induced mast cell degranulation was not worsened by L-NAME pretreatment. The findings suggest that NO could confer partial protection against stress-induced gastric ulcer formation; its activity is triggered off by the ulcerogenic mechanism of stress.
Key words. N^W-nitro-l-arginine methyl ester; nitric oxide; cold-restraint stress; mucosal ulcers; mast cells; rat stomachs.

The possibility that transmission at some nonadrenergic noncholinergic (NANC) neuroeffector junctions is mediated by nitric oxide (NO) arose from observations that NO was involved in the effects of nitrovasodilator drugs and that endothelium-derived relaxing factor (EDRF) was NO or a substance producing NO. There is now evidence for nitrergic innervation of smooth muscle in the gastrointestinal tract[1]. Recently, it has been suggested that NO plays a role in the maintenance of gastric mucosal integrity[2]; inhibition of NO formation worsens indomethacin-induced gastric mucosal damage[2] whereas NO may protect against ethanol-induced gastric lesions[3] in rats. In chronic renal failure, where peptic ulceration is known to occur frequently, the raised blood pressure is thought to be due to accumulation of an endogenous inhibitor of NO synthesis[4]; this suggests the possibility that NO deficiency could also be causally related to the high incidence of gastroduodenal mucosal damage.

The effect of NO on stress-induced gastric lesions is still unknown; clarification of this aspect is indeed important because NO is released in response to various stimuli, such as stress[5] where the stomach is one of the target organs. A centrally-mediated cholinergic element is thought to be the main pathological factor in stress-evoked ulceration because stress enhances the activity of the vagus[6], with consequent mucosal mast cell degranulation in rats[7–9]. Furthermore, vagal stimulation has been shown to release NO through a NANC pathway[10]. This communication reports some findings in rats on the influence of a N^W-nitro-l-arginine methyl ester (L-NAME), which inhibits NO formation, on gastric glandular mucosal ulceration induced by cold-restraint stress, and also on the associated mast cell degranulation.

Materials and methods

Female Sprague-Dawley rats, weighing 150–180 g, were used. They were fed a balanced pellet diet (Ralston Purina Co., USA) and housed in a room with controlled temperature (22 ± 1 °C) and humidity (65–70%). Solid food was withheld 48 h before exposure to restraint at 4 °C (stress) for 2 h[11]. The animals were initially housed in cages with raised wire floors, to prevent coprophagy, and given 8% sucrose in 0.2% NaCl w/v to drink. This drinking solution were removed 1 h before exposure to stress. L-NAME (Sigma) (3.125, 6.25, 12.5, 25, 50, 100 or 200 mg/kg), hexamethonium bromide (Pruiss, 20 mg/kg) or atropine sulphate (Sigma, 1 mg/kg) was freshly dissolved in 0.9% NaCl w/v solution (saline) and injected s.c. 20 min before experiments; hexamethonium/atropine and L-NAME were each injected into a different hind limb. Similar volumes of saline (2 ml/kg) were given by the same route to the controls. Each pretreatment group of rats was divided into two batches. One batch was restrained in individual close-fitting cylindrical cages at 4 °C and the other served as controls; these animals were left in their starvation cages in the room where they were normally housed.

At the end of 2 h, all rats were killed by a sharp blow on the head. Their stomachs were removed and opened along the greater curvature. Ulcer size was measured along the greatest length of the ulcer; five petechiae were considered equivalent to a 1 mm ulcer. The total of the lesion lengths divided by the number of rats in each batch was expressed as the mean ulcer index. Following lesion measurement, the glandular tissue was fixed in a freshly prepared 4% w/v lead acetate solution for 2 d. The method of staining tissue sections and counting mast cells was similar to that employed by Cho and

Ogle[11]. Data were analysed by the two-tailed Student's t-test, or one-way analysis of variance (ANOVA) when appropriate.

Results

The mean ulcer indices and glandular mucosal mast cell counts are shown in tables 1 and 2. In non-stressed rats, the mean ulcer index and mast cell population were unaffected by pretreatment s.c. with all doses of L-NAME (table 1A), or with hexamethonium or atropine (table 2A). The mucosal lesions seen in these non-stressed control animals were in the form of occasional petechiae. Cold-restraint stress for 2 h produced severe haemorrhagic ulcers and lowered the mast cell counts in the glandular mucosa. Pretreatment s.c. with L-NAME 12.5, 25, 50 or 100 mg/kg significantly increased stress-induced gastric ulcer size (table 1B, all $p < 0.05$); doses below 12.5 mg/kg were ineffective whereas 200 mg/kg did not worsen the mean ulcer index any further. The size of the stress-evoked ulcers was also dose-dependently aggravated by L-NAME (ANOVA; $F = 2.5$, $p < 0.05$). The mast cell counts which were decreased by stress were not lowered any further by the various pretreatment doses of L-NAME.

Hexamethonium 20 mg/kg or atropine 1 mg/kg, given s.c. 20 min beforehand, significantly antagonised stress-induced lesion formation (table 2B, $p < 0.001$) and mast cell degranulation (table 2B, $p < 0.05$). Pretreatment s.c. with either hexamethonium or atropine together with L-NAME 100 mg/kg also markedly antagonised the increased ulceration by the NO inhibitor as well as the mast cell degranulation evoked by stress. The degree of protection, as reflected by these two parameters, was not significantly different from that seen after pretreatment with hexamethonium or atropine alone.

Discussion

NO, identified as an EDRF[12], is released under basal conditions and also in response to various stimuli[5]. NANC inhibitory nerves have been described in the stomach[13], and have now been shown to be present in many parts of the gastrointestinal tract[14]. The present study suggests that endogenous NO may provide partial protection against gastric ulceration induced by cold-restraint stress, because its inhibition by L-NAME pretreatment intensifies lesion formation. It is likely that the doses of L-NAME (12.5–200 mg/kg s.c.) used in this investigation effectively inhibited NO synthesis, because similar doses are able to increase the mean blood pressure and lower the heart rate in anaesthetised rats observed over a period of 2 h (unpublished data). These findings are in accord with the observations and conclusions of Kiff et al.[15]. Depressed mucosal NO synthesis by L-NAME would result in unopposed vasoconstrictor activity by endothelin, to increase the susceptibility of the gastric mucosa to injury. Thus, a balance may exist between endothelium-derived contracting and relaxing factors which can markedly influence gastric mucosal integrity. It is possible that both NO and endothelin synthesis and release are increased by stress and that when NO is inhibited the balance is broken, and endothelin then exerts its ulcerogenic action.

There is little doubt that stress-induced gastric ulcer is a complex and multifactorial phenomenon. It is now generally accepted that there exists a balance between aggressive factors and defective mucosal mechanisms in

Table 1. The effects of pretreatment (20 min beforehand) with L-NAME on gastric ulcers and mucosal mast cell counts in stressed rats.

	Treatment (s.c.)	Dose	Ulcer index (mm)	Mast cell count (42 o.i.f.)
A.	No stress (unrestrained at 22 °C for 2 h)			
	Saline	2.000 ml/kg	0.03 ± 0.03	62 ± 6.2
	L-NAME	3.125 mg/kg	0.10 ± 0.10	62 ± 7.6
	L-NAME	6.250 mg/kg	0.03 ± 0.03	63 ± 7.1
	L-NAME	12.500 mg/kg	0.07 ± 0.07	64 ± 6.6
	L-NAME	25.000 mg/kg	0.10 ± 0.07	63 ± 5.2
	L-NAME	50.000 mg/kg	0.10 ± 0.05	61 ± 5.5
	L-NAME	100.000 mg/kg	0.10 ± 0.07	60 ± 4.5
	L-NAME	200.000 mg/kg	0.13 ± 0.07	62 ± 5.8
B.	Stress (restrained at 4 °C for 2 h)			
	Saline	2.000 ml/kg	5.98 ± 0.64**	41 ± 4.9*
	L-NAME	3.125 mg/kg	5.93 ± 0.68**	39 ± 6.4*
	L-NAME	6.250 mg/kg	6.20 ± 0.88**	41 ± 6.1*
	L-NAME	12.500 mg/kg	8.07 ± 0.63**+	42 ± 6.3*
	L-NAME	25.000 mg/kg	8.12 ± 0.72**+	43 ± 6.1*
	L-NAME	50.000 mg/kg	8.17 ± 0.73**+	40 ± 6.6*
	L-NAME	100.000 mg/kg	8.33 ± 0.75**+	41 ± 6.8*
	L-NAME	200.000 mg/kg	7.63 ± 0.76**	43 ± 5.8*

Values are expressed as means ± SEM of 6 rats in each group; o.i.f. = oil immersion fields (1000×); *$p < 0.05$; **$p < 0.001$ when compared to its corresponding non-stressed control in A; +$p < 0.05$ when compared to its own saline-injected control in B.

Table 2. The effects of pretreatment (20 min beforehand) with hexamethonium or atropine alone or with L-NAME on gastric ulcers and mucosal mast cell counts in stressed rats.

	Treatment (s.c.)	Dose	Ulcer index (mm)	Mast cell count (42 o.i.f.)
A.	No stress (unrestrained at 20 °C for 2 h)			
	Saline	2 ml/kg	0.07 ± 0.07	61 ± 4.7
	Hexamethonium	20 mg/kg	0.03 ± 0.03	64 ± 6.1
	Atropine	1 mg/kg	0.03 ± 0.03	60 ± 6.0
	Hexamethonium	20 mg/kg		
	+L-NAME	100 mg/kg	0.07 ± 0.04	62 ± 5.5
	Atropine	1 mg/kg		
	+L-NAME	100 mg/kg	0.10 ± 0.07	63 ± 5.9
B.	Stress (restrained at 4 °C for 2 h)			
	Saline	2 ml/kg	6.30 ± 0.58**	40 ± 3.6*
	Hexamethonium	20 mg/kg	0.66 ± 0.27*++	57 ± 5.5+
	Atropine	1 mg/kg	0.43 ± 0.15*++	58 ± 6.2+
	Hexamethonium	20 mg/kg		
	+L-NAME	100 mg/kg	0.9 ± 0.23*++	56 ± 5.2+
	Atropine	1 mg/kg		
	+L-NAME	100 mg/kg	0.73 ± 0.26*++	57 ± 4.9+

Values are expressed as means ± SEM of 6 rats in each group; o.i.f. = oil imersion fields (1000×); *$p < 0.05$; **$p < 0.001$ when compared to its corresponding non-stressed control in A;
+$p < 0.05$; ++$p < 0.001$ when compared to its own saline-injected control in B.

the stomach; any imbalance results in mucosal ulceration[16]. Glucocorticoids modulate the cytoprotective effect of adrenal catecholamines, and both adrenal glucocorticoids and catecholamines require an intact prostaglandin (PG) synthetic pathway for the expression of their cytoprotective properties[17]. However, oral administration of PG, which significantly elevates gastric PG levels, does not prevent stress-induced ulcers[18]; furthermore, an interaction between PG and NO is indeed unlikely[5].

Stimulation of peripheral dopamine receptors is primarily involved in the gastric cytoprotection[19], whereas bilateral microinjections of thyrotropin-releasing hormone (TRH) into the amygdala intensify gastric ulceration[20] in stress. However, L-NAME is reported only to antagonise NO synthesis[21] and there has been no report about the relationship between NO and dopamine receptors. The brain significantly influences the gastrointestinal tract[22], and stress increases activity in the central nervous system (CNS)[23], with consequent activation of the vagus which plays a major role in influencing the stomach[6].

The gastric mucosal mast cells are known to contribute significantly to stress-induced gastric ulcer formation[7–9]. CNS depression or cholinergic receptor blockade can prevent stress-induced mast cell degranulation and resulting gastric ulcer formation[24]. The fact that L-NAME intensifies stress-induced gastric mucosal damage without further decreasing the mast cell counts, suggests that the ulcer-worsening action of NO inhibition is not due to more mast cell degranulation through either a CNS or peripheral action. It has been shown that direct electrical vagal stimulation produces similar haemorrhagic ulcers in the glandular mucosa of rat stomachs[25]; stress-induced gastric ulcers thus appear to be mainly the result of cholinoceptor pathway activation. Stress as well as vagal nerve or local mucosal stimulation can release NO, which is also produced in basal conditions[4,5]. The inability of inhibition of NO synthesis by L-NAME to influence the small ulcer index in non-stressed rats, as well as the effectiveness of hexamethonium or atropine in antagonising stress-induced lesion formation and the ulcer-potentiating action of L-NAME, suggest that NO production and release are probably triggered off by vagal activation[6].

The present findings point to a localised action of NO in the stomach, rather than to an activity in the CNS. The antiulcer mechanism of NO is likely to be due to increased gastric mucosal blood flow[26], or its ability to produce stomach relaxation[10,27], or a combination of both effects. There is a third mechanism to be considered. The protective action against ulcers could also be partly or wholly due to NO normally antagonising an ulcerogenic factor, i.e. the vasoconstrictor effects of endothelin. The current preliminary results suggest some interesting possibilities, and more studies are needed.

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