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Role of central glutamate receptors, nitric oxide and soluble guanylyl cyclase in the inhibition by endotoxin of rat gastric acid secretion

[1]Eugenia García-Zaragozá, [1]M. Dolores Barrachina, [1]Lucrecia Moreno & *,[1]Juan V. Esplugues

[1]Department of Pharmacology, Faculty of Medicine, University of Valencia, Avda. Blasco Ibáñez 15, 46010 Valencia, Spain

1 This study examines the role of a central pathway involving glutamate receptors, nitric oxide (NO) and cyclic GMP in the acute inhibitory effects of low doses of peripheral endotoxin on pentagastrin-stimulated acid production.

2 Vagotomy or intracisternal (i.c.) microinjections of the NO-inhibitor, N^G-nitro-L-arginine methyl esther (L-NAME; 200 μg rat^{-1}) restored acid secretory responses in endotoxin (10 μg kg^{-1}, i.v.)-treated rats.

3 The acid-inhibitory effect of i.v. endotoxin (10 μg kg^{-1}, i.v.) was prevented by prior i.c. administration of the NMDA receptor antagonists, dizocilpine maleate (MK-801; 10 nmol rat^{-1}) and D-2-amino-5-phosphono-valeric acid (AP-5; 20 nmol rat^{-1}), or the AMPA/kainate antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 nmol rat^{-1}). However, the competitive metabotropic glutamate receptor antagonist (+)-α-methyl-4-carboxyphenylglycine (MCPG; 20–1000 nmol rat^{-1}) did not antagonize the effects of endotoxin.

4 I.c. administration of L-glutamate (0.1 nmol rat^{-1}) inhibited pentagastrin-stimulated gastric acid secretion. Coadministration with L-NAME (200 μg rat^{-1}) prevented the inhibition of gastric acid secretion by the aminoacid.

5 I.c. administration of 1H-[1,2,4]Oxazodiolo[4,3-a]quinoxalin-1-one (ODQ; 100 nmol rat^{-1}), a soluble guanylyl cyclase (sGC) blocker, reversed the hyposecretory effect of endotoxin.

6 I.c. administration of the cyclic GMP analogue 8-Bromoguanosine-3,5-cyclic monophosphate (8-Br-cGMP; 100–300 nmol rat^{-1}) reduced gastric acid production in a dose-dependent manner.

7 We conclude that central NMDA and AMPA/kainate receptors are involved in the acid inhibitory effect of peripherally administered endotoxin. This central pathway involves synthesis of NO, which acts on the enzyme sGC.

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Abbreviations: AP-5, D-2-amino-5-phosphono-valeric acid; 8-Br-cGMP, 8-Bromoguanosine-3,5-cyclic monophosphate; DNQX, 6,7-dinitroquinoxaline-2,3-dione; i.c., intracisternal; iGluR, ionotropic glutamate receptors; L-NAME, N^G-nitro-L-arginine methyl esther; MCPG, (+)-α-methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptors; MK-801, dizocilpine maleate; ODQ, 1H-[1,2,4]Oxazodiolo[4,3-a]quinoxalin-1-one

Introduction

Inhibition of gastric acid secretion is a characteristic of many infectious diseases and bacteraemia (Russell & Castro, 1987). Acid production is inhibited by the administration of doses of endotoxin (Barrachina *et al*., 1995b; Martínez-Cuesta *et al*., 1992; 1994) at levels similar to those present during moderate clinical endotoxemia (Hurley, 1995). The mechanism responsible for this effect involves a nervous reflex in which nitric oxide (NO) is released within brainstem nuclei implicated in the regulation of gastrointestinal function (Esplugues *et al*., 1996). Furthermore, this central nitrergic pathway is not only activated by endotoxins. Other hyposecretory stimuli such as the neuropeptides bombesin (Beltrán *et al*., 1999) and oxytocin (Esplugues *et al*., 1996), or stressful stimuli such as exposure to moderate hyperthermia or hypotension (Esplugues *et al*., 1996), all require this central synthesis of NO. Finally, the effects triggered by low doses of endotoxin do not seem to be limited only to modulation of acid responses, as shown by evidence for the involvement of a similar nitrergic pathway in changes of motility or adaptation to mucosal damage (Barrachina *et al*., 1995a; Martínez-Cuesta *et al*., 1997).

NO synthesis in the CNS is predominantly regulated by Ca^{2+} influx through receptor-operated channels, in particular following postsynaptic stimulation by the excitatory neurotransmitter glutamate (Bredt & Snyder, 1989; Garthwaite *et al*., 1989). In this way, NMDA receptors are of special importance, although AMPA, kainate and metabotropic glutamate receptors (mGluR) have also been implicated (Okada, 1992; Bhardwaj *et al*., 1997). Various mechanisms of action have been proposed for neuronally-produced NO, but the main physiological target or 'receptor' for NO seems to be the cytosolic enzyme, soluble guanylyl cyclase (sGC) (Bredt & Snyder, 1989; Southam & Garthwaite, 1991).

In the present study we have explored whether activation of glutamate receptors is involved in the endotoxin-activated NO-dependent neuronal pathway which leads to the inhibition of gastric acid secretion. In addition, the implication of sGC in the responses elicited by endotoxin has also been evaluated.

*Author for correspondence; E-mail: Juan.V.Esplugues@uv.es

Methods

General preparation

Male Sprague-Dawley albino rats (200–250 g body weight) were fasted for 16–20 h prior to the experiment and anaesthetized with urethane (1.5 g kg^{-1}, i.p.). A polyethylene tube was inserted in the trachea to ensure a patent airway and a jugular vein was cannulated. Two soft catheters were introduced into the gastric lumen through incisions in the cervical oesophagus and the duodenum and then it was flushed with 50–100 ml of saline to remove any solid content. Afterwards, the stomach lumen was continuously perfused with saline (1 ml min^{-1}) *via* the esophageal catheter. Following 1 h of stabilization, the gastric effluent was collected at 10 min intervals and acid production was measured by automatic titration (Radiometer Copenhagen, Denmark) of 7 ml aliquots of the perfusate to pH 7 with 0.01 M NaOH. Once acid secretion had remained constant for 60 min, animals received a bolus injection of pentagastrin (100 μg kg^{-1}, i.v.), which we will refer to as the control response. Only those rats producing more than 2 μEq H^+ 100 g^{-1} during the following 30 min period were used in further experiments. Thereafter, acid output was allowed to return to basal level. Following a period of 40 min, animals received the various treatments and acid production was elicited again by a similar dose of pentagastrin (100 μg kg^{-1}, i.v.). The acid output produced per animal in the initial 30 min of the control response (μEq H^+ 100 g^{-1} 30 min^{-1}) was considered as 100%, and any further measurement of acid production was compared to its respective control response and expressed as per cent of control. In each case, preparations were used for a single experiment only, with treatments allocated randomly.

Experimental protocol

Endotoxin (0.1, 1, 10 and 100 μg kg^{-1}) was administered i.v. 10 min prior to bolus injection of pentagastrin (100 μg kg^{-1}, i.v.). To analyse the role of central NO in the effects of endotoxin (10 μg kg^{-1}, i.v.), a group of rats received an intracisternal (i.c.) pre-treatment (10 min) with the NO synthesis inhibitor, L-NAME (N^G-nitro-L-arginine methyl esther, 200 μg rat^{-1}). In some cases, the cervical vagus was cut bilaterally. The role of endogenous glutamate in the inhibitory effects of endotoxin (10 μg kg^{-1}, i.v.) was evaluated by i.c. pre-treatment (10 min) with selective glutamate receptor antagonists. Four compounds were used: the non-competitive NMDA-receptor antagonist MK-801 (dizocilpine maleate; 10 nmol rat^{-1}); the competitive NMDA-receptor antagonist AP-5 (D-2-amino-5-phosphono-valeric acid; 20 nmol rat^{-1}); the AMPA/kainate receptor antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione; 10 nmol rat^{-1}) and the mGluR antagonist MCPG ([+]-α-methyl-4-carboxyphenylglycine; 20–1000 nmol rat^{-1}). In addition, in a further group of experiments, animals received a single i.c. injection of L-glutamate (0.1 nmol rat^{-1}) 10 min prior to the administration of pentagastrin. The implication of NO in the acid-responses elicited by L-glutamate was evaluated by i.c. co-administration of L-NAME (200 μg rat^{-1}). Finally, two experiments were performed in order to determine the role of endogenous cyclic GMP in acid inhibitory responses. In the first, animals received a single i.c. injection of the sGC inhibitor, ODQ (1H-[1,2,4]Oxazodiolo[4,3-a]quinoxalin-1-one; 50, 100 nmol rat^{-1}, i.c.) 10 min before i.v. endotoxin (10 μg kg^{-1}). In the second, rats were treated with the analogue of guanosine 3′5′ cyclic monophosphate (cyclic GMP), 8-Br-cGMP (8-Bromoguanosine-3,5-cyclic monophosphate; 30, 100, 300 nmol rat^{-1}) 10 min prior to administration of pentagastrin (100 μg kg^{-1}, i.v.).

Drugs

Urethane, pentagastrin, *Escherichia Coli* endotoxin (serotype 026:B6), L-NAME, MK-801, AP-5, DNQX, MCPG, L-glutamate, 8-Br-cGMP and ODQ were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Pentagastrin was first dissolved in a small amount of 0.01% NH_3. DNQX and ODQ were dissolved in 100% dimethylsulphoxide to give a 2 mM and 100 mM solution respectively. MCPG was dissolved in NaOH 1 mM to acquire a 100 mM solution. In each case drugs were finally dissolved in isotonic saline and buffered at physiological pH to the correct concentration. Unless otherwise mentioned, drugs or the respective vehicles were given in volumes of 1 ml kg^{-1} for i.v administration, or 10 μl rat^{-1} for i.c. injections.

Statistical analysis

All data were expressed as mean ± s.e.mean. Comparisons between groups were performed by ANOVA followed by a Newman-Keuls test, and in all cases a probability of $P<0.05$ or less was considered significant.

Results

In vehicle-treated animals ($n=9$), administration of pentagastrin (100 μg kg^{-1}, i.v.) induced a control acid secretory response which peaked at 20 min (5.1 ± 0.5 μEq H^+ 100 g^{-1} 10 min^{-1}), and returned to basal values within the next 40 min (1.1 ± 0.3 μEq H^+ 100 g^{-1} 10 min^{-1}). Following a subsequent period of 40 min, a second bolus of pentagastrin (100 μg kg^{-1}, i.v.) elicited a response which was 161.9 ± 19.2% of that of the control.

I.v. administration of endotoxin (0.1, 1, 10 and 100 μg kg^{-1}) significantly reduced, in a dose-dependent manner, the acid secretory effects of pentagastrin (34.1 ± 24.9, $n=3$, $P<0.05$; 54.3 ± 28.8, $n=3$, $P<0.01$; 54.5 ± 5.2, $n=7$, $P<0.01$; 41.9 ± 16.1, $n=4$, $P<0.01$; per cent of reduction respectively). These doses of endotoxin did not produce any changes in systemic arterial blood pressure or colonic temperature. The selection of the dose of 10 μg kg^{-1} of endotoxin (i.v.) used in subsequent experiments was based on these preliminary studies. In another group of experiments the acid inhibition elicited by 10 μg kg^{-1} of endotoxin (53.6 ± 6.6% $n=5$, $P<0.05$% of reduction) was not present in rats receiving a prior i.c. injection of 200 μg rat^{-1} of L-NAME, which exhibited an acid response similar to control ($n=5$). This dose of i.c. L-NAME did not modify the acid secretory effects of pentagastrin (100 μg kg^{-1}, i.v.) in vehicle-treated animals (195.5 ± 41.4, $n=4$% of response). When administered in vagotomized animals, endotoxin had no inhibitory effect on pentagastrin-stimulated acid output. Likewise, the effects of pentagastrin were not influenced by vagotomy (Table 1).

As shown in Figure 1, blockade of central NMDA-receptors with the antagonists MK-801 (10 nmol rat^{-1}, i.c.) and AP-5 (20 nmol rat^{-1}, i.c.), which exhibit different mechanisms of action, restored the acid secretory effects of pentagastrin in endotoxin-treated animals. When administered i.v., 10 nmol rat^{-1} of MK-801 had no effect on the inhibition of acid by endotoxin (75.9 ± 16.5% of control response, $n=3$;)

and, in order to reverse its effects, it was necessary to increase the dose up to 1000 nmol rat^{-1} (144.7±34.3% of control, $n=3$, $P<0.01$). Blockade of AMPA/kainate receptors with DNQX (10 nmol rat^{-1}, i.c.) prevented the endotoxin-induced reduction of gastric acid secretion (Figure 2), whereas blockade of central endogenous mGluR with the specific antagonist MCPG (20–1000 nmol rat^{-1}, i.c.) did not influence the inhibitory actions of peripheral endotoxin (Figure 3). Finally, blockade of both populations of ionotropic glutamate receptors (iGluR), or blockade of mGluR did not modify pentagastrin-stimulated acid secretion in vehicle-treated animals (Figures 1, 2 and 3). In a further group of experiments shown in Figure 4, the i.c. microinjection of L-glutamate (0.01 and 0.1 nmol rat^{-1}) reduced pentagastrin-stimulated gastric acid secretion. The highest dose of the aminoacid used in this study had no effect when injected i.v. The acid inhibitory effects of i.c. L-glutamate (0.1 nmol rat^{-1}) were prevented by co-administration of L-NAME (200 μg rat^{-1}) (Figure 4).

Blockade of central endogenous sGC by i.c. administration of ODQ (50–100 nmol rat^{-1}) prevented the inhibition of

Table 1 Reversal by vagotomy of the inhibition by endotoxin (10 μg kg^{-1}, i.v.) of pentagastrin (100 μg kg^{-1} i.v.) stimulated gastric acid secretion

	Vehicle (%)	n	*Endotoxin (%)*	n
Sham	139.6±11.8	9	81.8±2.9*	8
Vagotomy	137.5±19.1	5	135.1±25.0	6

Data is shown as per cent of control response (μEq H^+ 100 g^{-1} 30 min^{-1}) and expressed as mean±s.e.mean. Significance of difference from the vehicle treated group is shown as $*P<0.05$.

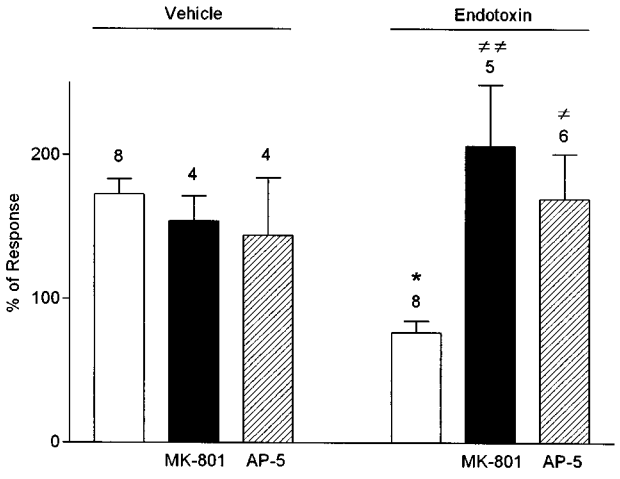

Figure 1 Blockade of NMDA receptors with MK-801 (10 nmol rat^{-1}, i.c.), a non-competitive NMDA receptor antagonist, and AP-5 (20 nmol rat^{-1}), a competitive NMDA receptor antagonist, prevented the inhibition by endotoxin (10 μg kg^{-1}, i.v.) of pentagastrin (100 μg kg^{-1}, i.v.) stimulated gastric acid secretion. Each column shows mean±s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$; significant difference from the endotoxin treated group is shown by $\neq P<0.05$ and $\neq\neq P<0.01$. Numbers above the columns indicate the number of animals used.

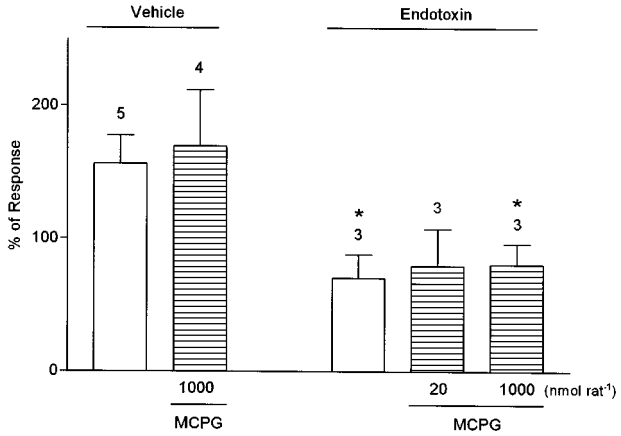

Figure 3 Blockade of metabotropic glutamate receptors by pretreatment with MCPG (20, 1000 nmol rat^{-1}, i.c.) did not influence the inhibition by endotoxin (10 μg kg^{-1}, i.v.) of pentagastrin (100 μg kg^{-1}, i.v.) stimulated gastric acid secretion. Each column shows mean±s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$. Numbers above the columns indicate the number of animals used.

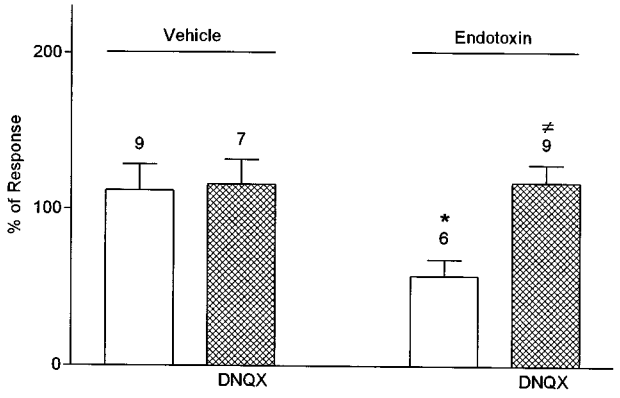

Figure 2 Pretreatment (i.c.) with the AMPA/kainate receptor antagonist DNQX (10 nmol rat^{-1}) prevented the inhibition by endotoxin (10 μg kg^{-1}, i.v.) of pentagastrin (100 μg kg^{-1}, i.v.) stimulated acid secretion. Each column shows mean±s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$; significant difference from the endotoxin treated group is shown by $\neq P<0.05$. Numbers above the columns indicate the number of animals used.

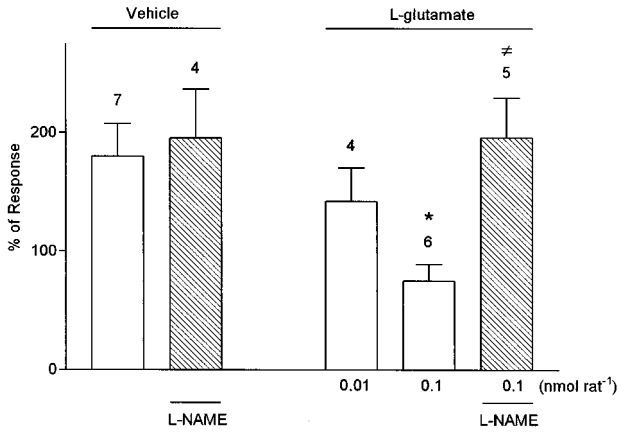

Figure 4 Inhibition by L-glutamate (i.c.) of pentagastrin (100 μg kg^{-1}, i.v.) stimulated acid secretion. Co-administration of L-NAME (200 μg rat^{-1}, i.c.) prevented the inhibitory effects of L-glutamate. Each column shows mean±s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$; significant difference from the L-glutamate (0.1 nmol rat^{-1}, i.c.) treated group is shown by $\neq P<0.05$. Numbers above the columns indicate the number of animals used.

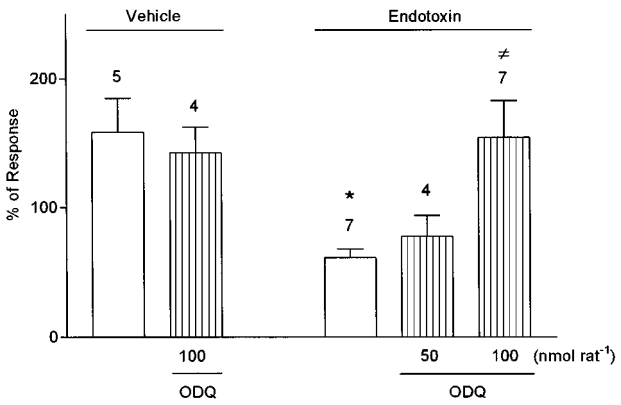

Figure 5 Blockade of endogenous sGC with ODQ (50, 100 nmol rat^{-1}, i.c.) prevented the inhibition by endotoxin (10 $\mu g\ kg^{-1}$, i.v.) of pentagastrin (100 $\mu g\ kg^{-1}$, i.v.) stimulated acid secretion. Each column shows mean ± s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$; significant difference from the endotoxin treated group is shown by $\neq P<0.05$. Numbers above the columns indicate the number of animals used.

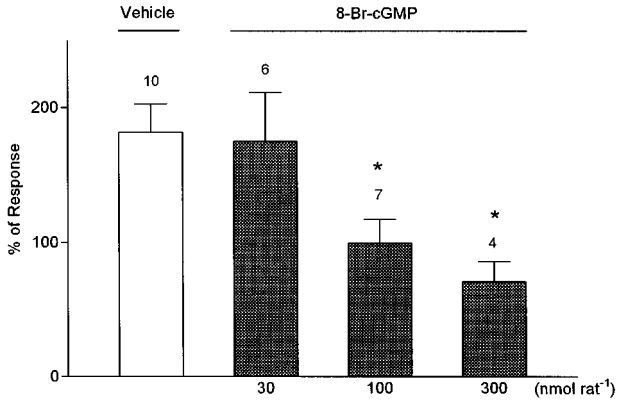

Figure 6 Acid secretion stimulated by pentagastrin (100 $\mu g\ kg^{-1}$, i.v.) was dose-dependently inhibited by i.c. administration of the cell permeable cyclic GMP analogue, 8-Br-cGMP. Each column shows mean ± s.e.mean; significant difference from the vehicle treated group is shown by $*P<0.05$. Numbers above the columns indicate the number of animals used.

pentagastrin-stimulated gastric acid secretion by endotoxin (Figure 5). However, ODQ alone did not alter gastric acid secretion in vehicle-treated animals. As shown in Figure 6, i.c. administration of the analogue of cyclic GMP, 8-Br-cGMP (30, 100, 300 nmol rat^{-1}), resulted in a dose-dependent inhibition of pentagastrin induced gastric acid secretion.

Discussion

Functional (Panico *et al*., 1995; Krowicki *et al*., 1997), immunohistochemical (Esplugues *et al*., 1996) and electrochemical (Beltrán *et al*., 1999) studies provide evidence that activation of an L-arginine/NO pathway in the brain is involved in the control of various gastric functions. This idea is further supported by the present study, which shows that i.c. pre-treatment with the inhibitor of NO synthesis L-NAME prevented the inhibitory effect of low doses of peripherally administered endotoxin on pentagastrin-induced acid secretion. In addition, these results expand previous observations that synthesis of NO in the brain was required for endotoxin to inhibit secretion induced by centrally mediated nervous stimulants such as gastric distension (Martínez-Cuesta *et al*., 1994; Barrachina *et al*., 1995b). They also suggest that this inhibitory nitrergic pathway is effective on acid-responses elicited in the periphery, such as those of pentagastrin (Lloyd & Haile, 1994). Vagotomized rats exhibited a normal secretory response to pentagastrin, thus confirming the peripheral nature of the action of this secretagogue. However, the inhibitory action of endotoxin was dependent on the integrity of the vagus nerve as demonstrated by its absence in animals with bilateral vagotomy. The vagus nerve contains both efferent and afferent fibres and, although the experimental design used here does not discriminate between them, there is previous experimental evidence suggestive of a potential role for both in this acid inhibitory mechanism. Thus, a vagal efferent pathway mediates the acid inhibitory actions of bombesin, a neuropeptide that activates a cerebral nitrergic pathway similar to that described here (Beltrán *et al*., 1999). In addition, it has been reported that peripheral endotoxin induces fos immunoreactivity in primary afferent neurons of the vagus (Gaykema *et al*., 1998). Taking into account that endotoxin crosses the blood-brain barrier with difficulty (Rowley *et al*., 1956; Dascombe & Milton, 1979), it is feasible that this particular acid inhibitory stimuli acts, directly or indirectly, on vagal afferent fibres. Such fibres would process the information to the brain, where vagal motor neurons would then be activated in a process involving the synthesis of NO.

Previous studies have shown that peripheral endotoxin induces increases in the levels of glutamate in central nuclei related to gastrointestinal function (Mascarucci *et al*., 1998). Glutamate is the main EAA neurotransmitter in the brain and the principal stimulant of central NO production. It is also involved in various physiological and pathophysiological processes of the CNS (Dingledine *et al*., 1999). Two different types of glutamate receptors have been characterized in the CNS: mGluR coupled to G proteins (Conn & Pin, 1997) and iGluR coupled to voltage operated calcium channels, the latter including the NMDA and the AMPA/kainate receptor subtypes (Dingledine *et al*., 1999). In the present study we show that blockade of glutamate receptors did not modify pentagastrin-stimulated acid secretion in vehicle-treated animals. This disputes a possible tonic control by central glutamate on the production of acid, and is in contrast with evidence for the existence of this tonic influence on other gastric functions such as motility (Bongianni *et al*., 1998). However, i.c. administration of two different NMDA receptor antagonists, MK-801 and AP-5, prevented endotoxin-induced inhibition of acid secretion. Similar results were obtained after blockade of AMPA/kainate receptors by pre-treatment with DNQX, but not following blockade of mGluR with MCPG. This suggests that endogenous glutamate acting specifically on iGluR is implicated in the inhibition by endotoxin of pentagastrin-stimulated acid secretion. There are reports implicating both populations of iGluR in other CNS responses, particularly in primary visceral afferent transmission, such as in baroreceptor signalling in the nucleus tractus solitarius (Chen & Bonham, 1998). Despite previous evidence of a peripheral role for glutamate in the inhibition of gastric secretion (Tsai *et al*., 1994), our results support a central action of this aminoacid in the acid effects of endotoxemia. Thus, doses of MK-801 that were effective i.c. had no effect when administered i.v., and it was necessary to increase such i.v. doses 100 fold to reverse the effects of endotoxin. The central nature of the effects of glutamate was also implied in the finding that exogenous i.c. administration of the aminoacid

reduced pentagastrin-stimulated secretion at doses that had no effect when injected i.v. and which did not produce any apparent cardiovascular or respiratory changes. Co-administration of L-NAME prevented the inhibitory effect of exogenous glutamate, further reinforcing the idea that central NO mediates the actions of this aminoacid and that it plays a role in the central modulation of gastric function.

Once synthesised, the main target of NO seems to be the heme group of sGC, to which it binds in order to promote the synthesis of the second messenger cyclic GMP (Moncada *et al*., 1991). In order to characterize the influence of central cyclic GMP in the control of gastric acid secretion we have modulated pharmacologically the levels of this intracellular messenger by: (i) blockade of endogenous sGC with ODQ (Garthwaite *et al*., 1995), and (ii) exogenous administration of the cyclic GMP analogue, 8-Br-cGMP (Kobzik *et al*., 1994). When administered alone, i.c. ODQ did not modify acid production; however, it reversed in a dose-dependent manner the inhibition by endotoxin of pentagastrin-induced acid secretion. Although this finding excludes a role for sGC in the effects of pentagastrin, it suggests that an increase in the activity of central sGC is involved in the acid-inhibitory effects of endotoxin. In addition, the fact that i.c. administration of 8-Br-cGMP dose-dependently reduced pentagastrin-stimulated acid secretion supports the idea that an endotoxin-induced increase in cyclic GMP levels in the brain mediates its inhibition of acid secretion. Our findings prompt two further questions: the possibility that other substances which increase the level of cyclic GMP also inhibit gastric acid secretion, and the role of this mediator in other central inhibitory pathways of gastrointestinal functions.

In conclusion, the present results suggest that a central glutamate/NO/cyclic GMP pathway is involved in endotoxin-induced inhibition of gastric acid secretion. Peripheral endotoxin activates a central pathway in which endogenous glutamate is involved. This aminoacid acts on ionotropic receptors and elicits the endogenous production of NO, which subsequently activates sGC.

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