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Central GABA-A receptors exert a tonic inhibitory control on gastric pepsinogen secretion in anaesthetized rats

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1 The purpose of the present study was to analyse the role played by central gamma-aminobutyric acid (GABA-A) receptors in the regulation of gastric basal pepsinogen secretion in anaesthetized rats.

2 The central, but not parenteral, administration of the GABA-A receptor antagonist bicuculline or SR-95531 caused a significant and dose-dependent increase in basal pepsinogen secretion without changes in acid output. The stimulant effect exerted by bicuculline was prevented by atropine or pirenzepine, but not by vagotomy.

3 The central, but not parenteral, administration of the GABA-A receptor agonist muscimol or THIP induced a significant and dose-dependent stimulation of both basal pepsinogen and acid secretion. The excitatory effect exerted by muscimol was prevented by atropine, pirenzepine, or pretreatment with omeprazole, but not by vagotomy.

4 These results suggest that central GABA-A receptors mediate a tonic inhibitory control on gastric pepsinogen secretion, while their phasic activation leads to an excitatory effect on acid output. However, the agonist-induced pepsigogue action appears to be generated peripherally as an indirect consequence of the increase in acid secretion.

5 It is also suggested that central GABA-A receptors affect the gastric secretory functions through non-vagal pathways that are sensitive to the blockade of peripheral cholinergic receptors.

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Introduction

Several neurotransmitters and hormones have been shown to affect gastric acid secretion significantly, through the activation of excitatory or inhibitory pathways at the level of the central nervous system (Taché, 1987). In spite of this, scarce attention has been paid to the possible influence exerted by central neurotransmitters on the regulation of gastric pepsinogen secretion. Interestingly, such disregard appears to be mainly encouraged by the observation that pepsin release often parallels the secretion of acid, thus implying the involvement of common mechanisms (Basson, Adrian & Modlin, 1988). Indeed, pepsinogen secretion generally increases in several preparations, in response to agents that stimulate acid secretion (Hersey, 1987), and decreases *in vivo* following the administration of H_2 receptor antagonists (Berstad, Ryning, Kolstad & Frislid, 1981). However, in contrast with these findings it has been shown unequivocally that pepsin and acid secretion may vary independently in several *in vivo* and *in vitro* experimental models (Basson *et al.*, 1988; Magee, 1989).

Gamma-aminobutyric acid (GABA) is a well-established inhibitory neurotransmitter of the central nervous system, where its actions are mediated through the activation of two distinct receptors, named GABA-A and GABA-B (Bowery *et al.*, 1984). Several lines of evidence indicate that central GABAergic pathways play a significant role in the regulation of gastric acid secretion (Levine, Morley, Kneip, Grace & Silvis, 1981; Bhargava, Gupta & Gupta, 1985; Del Tacca, Blandizzi & Bernardini, 1990). In particular, it was shown that the activation of bicuculline-sensitive GABA-A receptors increases basal acid secretion in anaesthetized rats, whereas phaclofen-sensitive GABA-B receptors mediate an inhibition of vagally stimulated acid output (Del Tacca *et al.*, 1990; Blandizzi, Bernardini, Natale & Del Tacca, 1991). Nevertheless, the possibility that the central GABAergic system could also participate in the regulation of gastric pepsinogen secretion has not yet been subject to investigation. In addition, based on the evidence that parenterally administered atropine suppressed the increase in acid secretion mediated by central GABA-A receptors, it was suggested that vagal cholinergic pathways might be involved in this excitatory effect (Levine *et al.*, 1981). However, conclusive evidence accounting for this proposed mechanism has not been provided.

The aim of the present study was to investigate the role played by central GABA-A receptors in the regulation of gastric basal pepsinogen secretion in anaesthetized rats, as well as to understand better the mechanisms underlying the gastric hypersecretory effects induced by the activation of central GABA-A receptors.

Methods

Animals

The experiments were carried out on male Wistar rats, weighing about 200 g. The rats were fed standard laboratory chow and tap water *ad libitum*, and were not used for at least one week after their delivery to the laboratory. The animals were housed six to a cage in temperature-controlled rooms on a 12-h light cycle at 22–24°C and 50–60% humidity. Their care and handling were in accordance with the provisions of the European Community Council Directive 86–609, recognized and adopted by the Italian Government.

Three to four days before the experiments, a chronic cannula was implanted into the lateral ventricle of the brain, during a short anaesthesia induced with pentobarbital sodium (30–40 mg kg^{-1}) given intraperitoneally (i.p.), as previously described by Caulfield, Clover, Powers & Savage (1983).

Twenty-four hours before the experiments, the animals were maintained in single cages provided with wire net bottoms in order to prevent coprophagy, and they were deprived of food. Free access to water *ad libitum* was allowed until one hour before the experiment. At the time of the experiment, the drugs under investigation were administered by intracerebroventricular (i.c.v.) or intravenous (i.v.) route. In the former case, the drug solutions (2.5 μl) were injected through the cannula using a 10-μl Hamilton microsyringe, followed by a 2.5 μl of saline solution (154 mM NaCl). The solutions were injected within 20 s. In agonist–antagonist interaction experiments, the antagonist was administered 10 min before

agonist. All drugs were tested using a single dose technique.

Stomach-perfused rats

Continuous perfusion of the rat stomach *in situ* was carried out following the procedure developed by Ghosh & Schild (1958). The animals were anaesthetized with urethane (1.2 g kg^{-1} i.p.), and a polyethylene catheter was introduced into the oesophagus and advanced as far as 5 mm beyond the gastro-oesophageal junction. A second catheter was introduced into the duodenum and pushed forward until its tip was about 5 mm beyond the pylorus. The stomach lumen was perfused continuously with 154 mM NaCl solution at 37°C, at a rate of 1 ml min^{-1}, and 15-min effluent fractions were collected. The effluent samples were used for the quantitative evaluation of both pepsinogen and acid secretion. Rectal temperature was monitored and maintained between 37 and 39°C with an infrared lamp.

A series of experiments was performed on rats whose vagus nerves were carefully separated from the carotid arteries and cut at the cervical level at the time of the experiment. The proximal and distal ends of both vagus nerves were crushed in order to prevent efferent stimulation of the distal trunks (Blandizzi, Bernardini, Natale, Martinotti & Del Tacca, 1992). Additional experiments were carried out in animals pretreated with the gastric proton pump-blocker omeprazole (30 mg kg^{-1} i.p.) 90 min before starting the collection of the gastric effluent fractions.

Evaluation of gastric pepsinogen and acid secretion

Pepsin levels in the gastric effluent were determined by the method of Berstad (1970) with minor modifications. Briefly, 0.5 ml of gastric effluent was added to tubes containing 2 ml of 2.5% bovine haemoglobin and 0.5 ml of 0.3 N HCl. Samples were then incubated for 10 min at 37°C and the reaction was stopped by addition of 5 ml 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by a Perkin–Elmer Spectrophotometer (Norwalk, CT, USA). The results were compared with a standard curve, generated in an identical manner using known amounts of porcine pepsin (1 $\mu g = 3$ peptic units). Data were expressed as μg of pepsin/15 min.

The acidity in the gastric perfusate was measured with an Autotitrator pH Meter (PHM85, Radiometer, Copenhagen, Denmark) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH. Data were expressed as μEqH^+/15 min.

Drugs

The following drugs and reagents were used: muscimol, (-)-bicuculline methiodide, urethane ethyl carbamate, crystallized porcine pepsin, lyophilized bovine haemoglobin (Sigma, St Louis, MO, USA); pirenzepine dihydrochloride (Boehringer, Ingelheim, Germany); atropine sulphate (BDH Chemicals Ltd, Poole, England), 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl) pyridazinium bromide (SR-95531), 4,5,6,7-tetrahydroisoxazol [5,4-c]pyridin-3-ol hydrochloride (THIP) (RBI, Natick, MA, USA); omeprazole (Malesci, Florence, Italy); and pentobarbital sodium (Clin-Midy, Paris, France). Other reagents were of analytical grade.

Statistics

Results are given as means ± SEM. The significance of differences was evaluated using Student's *t*-test for unpaired data and *P* values lower than 0.05 were considered significant; *n* indicates the number of experiments.

Results

In control animals with intact vagus nerves, following a 30-min stabilization period, both basal pepsinogen and acid secretion remained nearly unaltered for up to 4 h, ranging from 71.5 ± 11.7 to 88.2 ± 13.5, and from 3.5 ± 0.7 to 4.4 ± 1.0, respectively ($n = 6$). Under these conditions, atropine (2.5 mg kg^{-1} i.v., $n = 6$), pirenzepine (10 mg kg^{-1} i.v., $n = 6$), and omeprazole (30 mg kg^{-1} i.p., $n = 6$), each given alone, were without effect on basal pepsinogen and acid output (not shown).

When tested on animals with intact vagus nerves, the GABA-A receptor antagonist bicuculline (0.1, 0.3, 1 and 3 μg/rat i.c.v., $n = 6$ for each dose) caused a significant and dose-de-

pendent increase in basal pepsinogen secretion, the maximal stimulatory effect occurring at the dose of 1 μg/rat (Fig. 1A). When tested at the dose of 0.03 μg/rat i.c.v. ($n = 6$), bicuculline failed to modify the basal pepsinogen output (not shown). The excitatory action of bicuculline was mimicked by the GABA-A receptor antagonist SR-95531 (1, 3 and 9 μg/rat i.c.v., $n = 6$ for each dose), which induced a maximal increment of the basal pepsinogen output at the dose of 3 μg/rat (Fig. 2A). In contrast, both i.c.v. bicuculline and SR-95531 did not significantly modify basal acid secretion in the same animals (Figs 1B and 2B). Under the same experimental conditions, the stimulatory effect exerted by bicuculline (1 μg/rat i.c.v.) on basal pepsinogen output was completely prevented by pretreatment with atropine (2.5 mg kg^{-1} i.v., $n = 6$) or pirenzepine (10 mg kg^{-1} i.v.; $n = 6$), whereas it was not significantly affected by bilateral cervical vagotomy ($n = 6$) (Fig. 3). When bicuculline (0.03, 0.1, 0.3, 1 and 3 μg/rat i.c.v.; $n = 4$ for each dose) or SR-95531 (1, 3 and 9 μg/rat i.c.v.; $n = 4$ for each dose) was administered by the intravenous route, no significant variations were detected in both pepsinogen and acid secretion at all the doses tested (not shown).

In animals with intact vagus nerves, the GABA-A receptor agonist muscimol (0.3, 1 and 3 μg/rat i.c.v.; $n = 6$ for each dose) induced a significant and dose-dependent stimulation of basal gastric peptic secretion, the maximal hypersecretory effect being observed at the dose of 1 μg/rat (Fig. 4A). The stimulant action exerted by muscimol on pepsinogen secretion was mimicked by the GABA-A receptor agonist THIP (4.5, 9 and 18 μg/rat i.c.v.; $n = 6$ for each dose), which caused a maximal excitatory effect at the dose of 9 μg/rat (Fig. 5A). Contrary to the results obtained by central injection of bicuculline or SR-95531, both muscimol and THIP induced a significant and dose-dependent increase in acid secretion that paralleled the stimulant actions exerted by these drugs on gastric pepsinogen output (Figs 4B and 5B). In the presence of the same conditions, bicuculline (0.03 μg/rat i.c.v., $n = 6$) did not affect the gastric hypersecretory effects elicited by muscimol (0.3 μg/rat i.c.v., $n = 6$) (not shown). In addition, both the peptic and acid hypersecretory effects evoked by muscimol (1 μg/rat i.c.v.) were fully inhibited by pretreatment with omeprazole (30 mg kg^{-1} i.p.; $n = 6$), atropine (2.5. mg kg^{-1} i.v., $n = 6$) or pirenzepine (10 mg kg^{-1} i.v.; $n = 6$), whereas they were not significantly influenced by bilateral cervical vagotomy ($n = 6$) (Fig. 6). Following the administration of muscimol (0.3, 1 and 3 μg/rat; $n = 4$ for each dose) or THIP (4.5, 9 and 18 μg/rat; $n = 4$ for each dose) by intravenous route, the levels of both pepsinogen and acid secretion did not vary significantly from basal levels (not shown).

Discussion

Previous evidence consistently indicates the major role that central GABA-A and GABA-B receptors play in the modulation of gastric acid secretion in the presence of different experimental conditions (Levine *et al.*, 1981; Del Tacca *et al.*, 1990; Blandizzi *et al.*, 1991). The results obtained in the present study further support the view that central GABA-A receptors exert an excitatory control on acid output and also provide new evidence for the involvement of central GABAergic pathways in the regulation of gastric pepsinogen release.

Under the present experimental conditions, both bicuculline and SR-95531, two selective GABA-A receptor antagonists endowed with different structures (Krogsgaard-Larsen, Hjeds, Falch, Jorgensen & Nielsen, 1988), were able to dose-dependently stimulate basal pepsinogen output only when injected by i.c.v. and not i.v. route. On the other hand, the same drugs were devoid of any effect on the acid component of gastric secretion, irrespective of the administration route used. Taken together, these findings suggest that, in the presence of a pharmacological depression of the central nervous system induced by anaesthesia with urethane, the central GABAergic pathways exert a tonic inhibitory control on gastric pepsinogen secretion through the activation of central GABA-A receptors, and that such a modulatory action is completely independent of the acid secretory profile.

The lack of significant effects of bicuculline or SR-95531 on acid output suggests that the central GABA-A receptors are not tonically involved in the modulation of this gastric secretory function. However, a significant increase in acid secretion was observed following

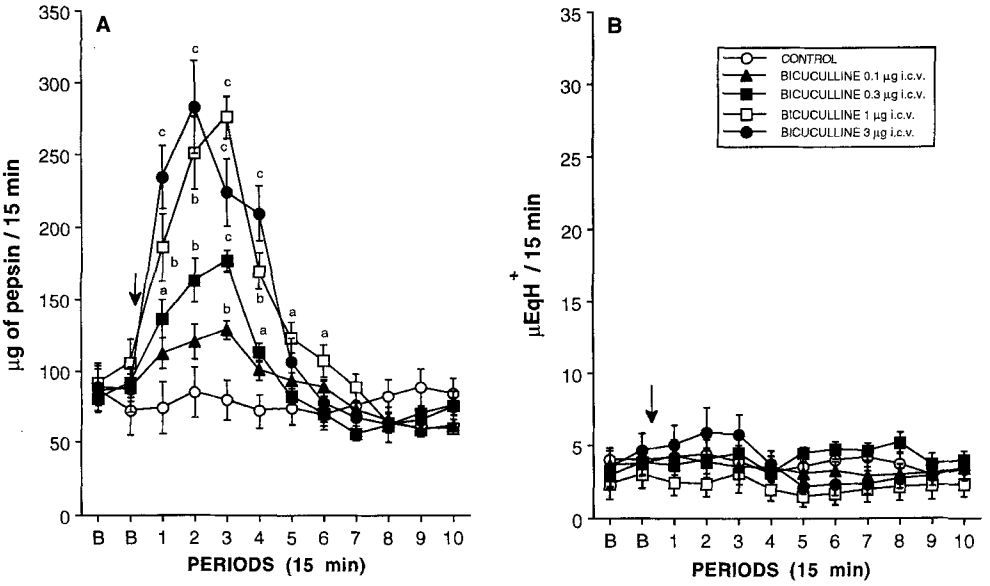

Fig. 1. Effects of bicuculline (0.1, 0.3, 1 and 3 µg/rat i.c.v.) on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The single arrow indicates the time of bicuculline administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

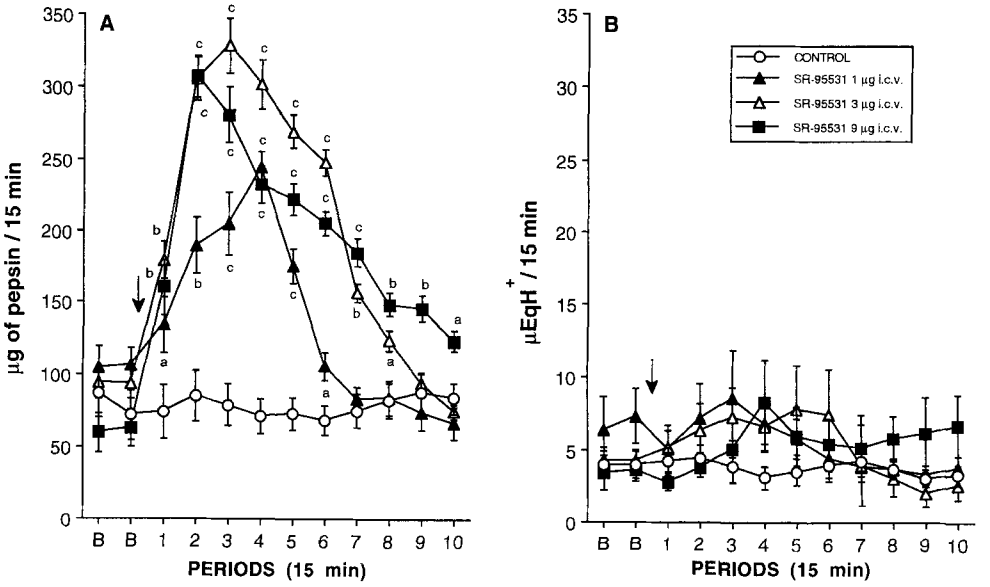

Fig. 2. Effects of SR-95531 (1, 3 and 9 µg/rat i.c.v.) on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The single arrow indicates the time of SR-95531 administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

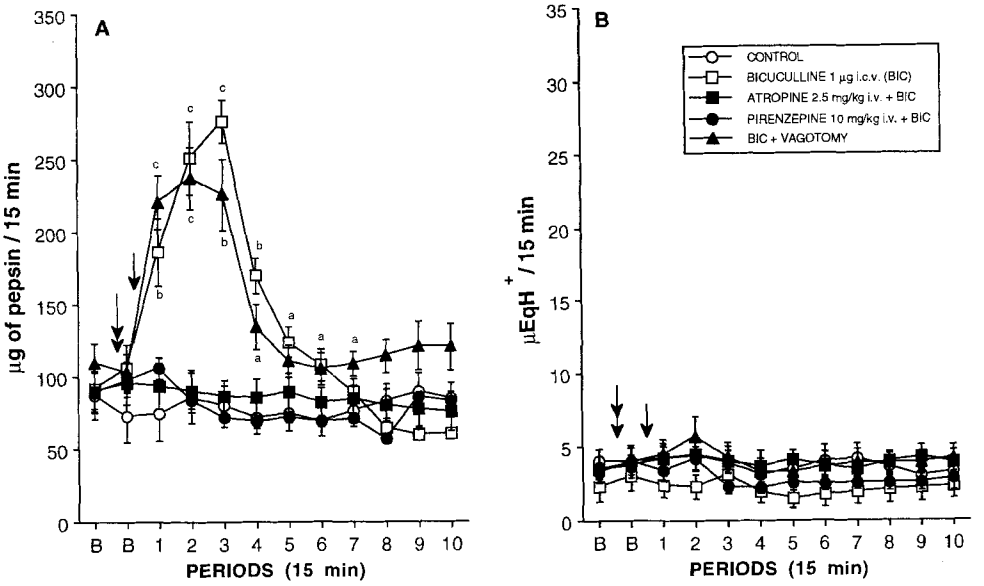

Fig. 3. Effects of bicuculline (1 µg/rat i.c.v.), atropine (2.5 mg kg^{-1} i.v.) plus bicuculline (1 µg/rat i.c.v.), pirenzepine (10 mg kg^{-1} i.v.) plus bicuculline (1 µg/rat i.c.v.), or bicuculline (1 µg/rat i.c.v.) in the presence of bilateral cervical vagotomy on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The double arrow indicates the time of atropine or pirenzepine administration, and the single arrow indicates the time of bicuculline administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

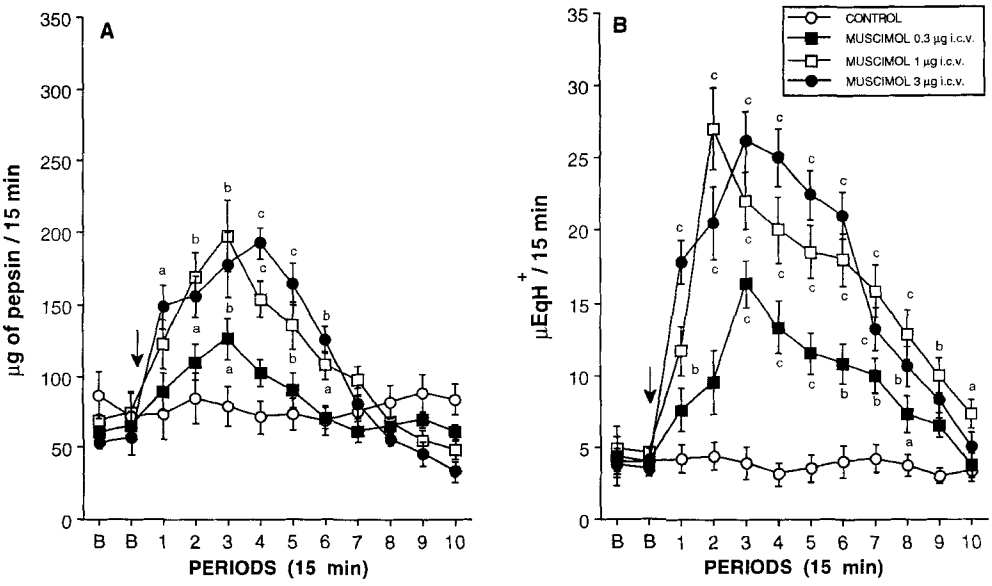

Fig. 4. Effects of muscimol (0.3, 1 and 3 µg/rat i.c.v.) on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The single arrow indicates the time of muscimol administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

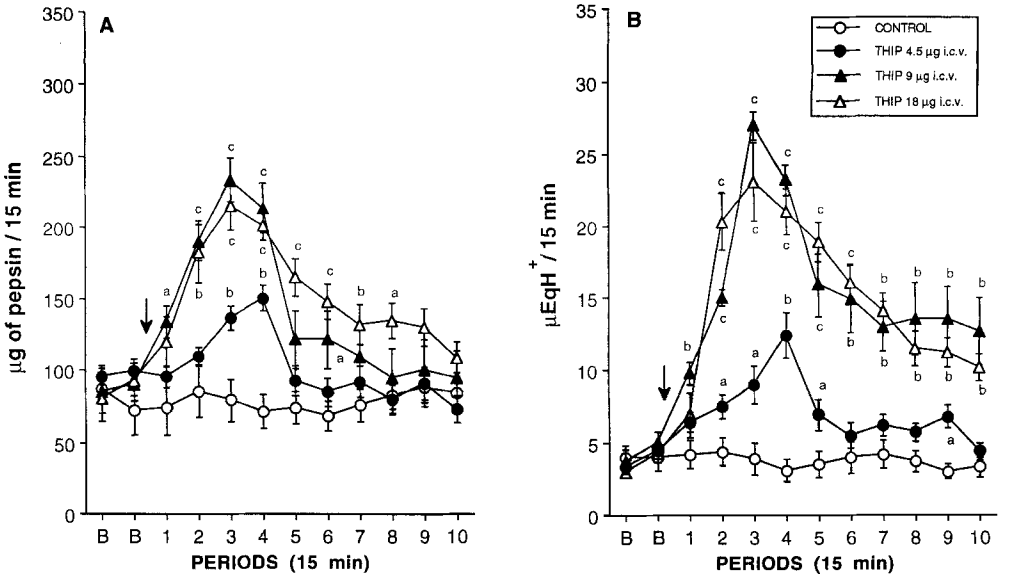

Fig. 5. Effects of THIP (4.5, 9 and 18 µg/rat i.c.v.) on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The single arrow indicates the time of THIP administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

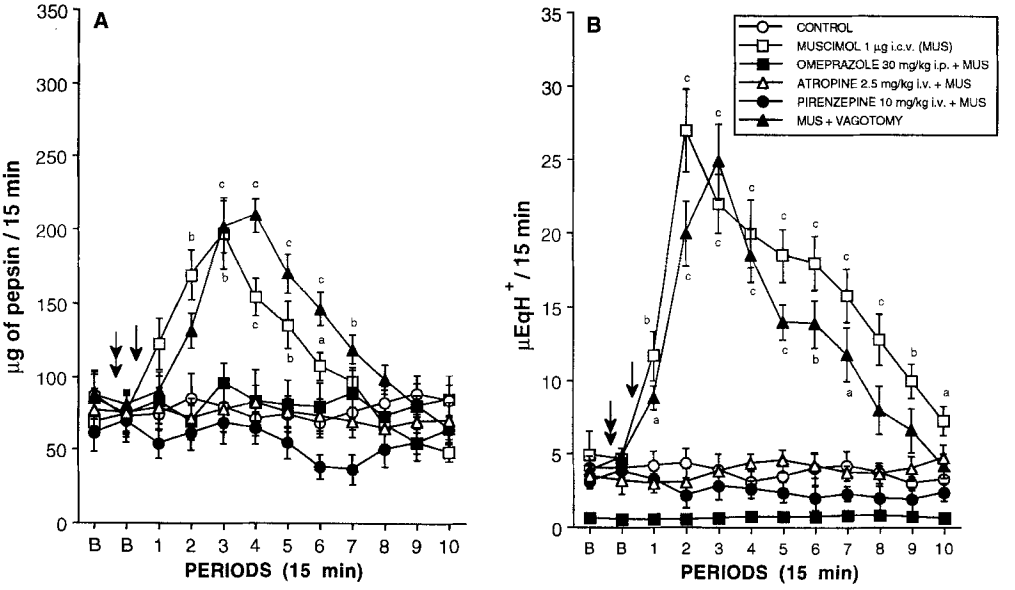

Fig. 6. Effects of muscimol (1 µg/rat i.c.v.), omeprazole (30 mg kg^{-1} i.p.) plus muscimol (1 µg/rat i.c.v.), atropine (2.5 mg kg^{-1} i.v.) plus muscimol (1 µg/rat i.c.v.), pirenzepine (10 mg kg^{-1} i.v.) plus muscimol (1 µg/rat i.c.v.), or muscimol (1 µg/rat i.c.v.) in the presence of bilateral cervical vagotomy on basal pepsinogen (A) and acid (B) secretions in anaesthetized stomach-perfused rats. Each point represents the mean value obtained from six animals ± SEM (vertical lines). The double arrow indicates the time of atropine or pirenzepine administration, and the single arrow indicates the time of muscimol administration. B indicates basal value. Significant difference from control values: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

the i.c.v. administration of the GABA-A receptor agonists muscimol and THIP, indicating that central GABA-A receptors can be phasically recruited to evoke an enhancement of basal acid output.

Overall, our data support the view that central GABA-A receptors play a differential role in the regulation of gastric secretory functions, which consists of a tonic inhibition of peptic secretion and a phasic stimulation of acid output. In respect of this, examples of differential regulation of pepsin and acid secretion by the same agents have already been reported (Basson *et al.*, 1988; Magee, 1989). In particular, gastrin or cholecystokinin could increase the acid secretin from Heidenhain pouch dogs without affecting pepsin output (Dutt & Magee, 1972; Magee & Dutt, 1972), whereas secretion was found to stimulate pepsin secretion, but not acid, in humans (Berstad & Petersen, 1970), dogs (Nakajima, Nakamura & Magee, 1969), and cats (Beswick, Braganza & Howat, 1979). More recently, *in vitro* experiments showed that histamine increases basal acid secretion from guinea-pig fundic mucosal sheets without altering pepsinogen secretion (Basson *et al.*, 1988).

In the present study, it was also observed that the central injection of both muscimol and THIP led to a significant increase in pepsinogen secretion that paralleled the concomitant increment of acid output. This finding is in apparent contrast with the conclusion that in anaesthetized rats the central GABA-A receptors are tonically activated to mediate an inhibitory modulation of gastric peptic secretion. Indeed, according to the results obtained in the presence of bicuculline and SR-95531, GABA-A receptor agonists would be expected not to affect, or possibly to inhibit, basal pepsinogen output. Several agents, known to stimulate acid secretion, also exert an indirect pepsigogue action depending on the ability of gastric luminal acid to enhance pepsinogen output through the activation of local gastric nervous reflexes (Johnson, 1972; Hersey, 1987; Smith & Torres, 1990). However, both *in vitro* and *in vivo* experiments showed that the benzimidazole derivative omeprazole proved able to discriminate between direct or indirect (i.e. acid-mediated) stimulation of pepsin secretion (Basson *et al.*, 1988; Blandizzi, Martinotti, Natale, Carignani & Del Tacca, 1994), owing to its unique property of inhibiting acid secretion through a blockade of gastric H^+:K^+-ATPase without affecting cell receptors or transduction pathways (Clissold & Campoli-Richards, 1986). In addition, we observed that acute treatment with omeprazole alone was without significant effect on pepsinogen secretion, while our observations on the abolition of the acid output were in full agreement with data reported by other authors (Clissold & Campoli-Richards, 1986; McTavish, Buckley & Heel, 1991). On these bases, the present results, showing that both the acid and peptic hypersecretory effects induced by muscimol were completely prevented by pretreatment with omeprazole, suggest that the pepsigogue action of muscimol does not directly depend on the activation of central GABA-A receptors; rather it represents an acid-mediated peripheral response.

Agonist–antagonist interaction experiments were quite difficult to perform in this study, since both GABA-A receptor agonists and antagonists, given alone, exerted stimulant effects on pepsinogen secretion, although through the activation of different mechanisms. In particular, when tested at a dose not influencing pepsinogen output *per se*, bicuculline was not able to modify the hypersecretory effects induced by muscimol. This failure seems to be attributable to the great disproportion in the dose ratio of bicuculline versus muscimol (1:10). Indeed, previous studies showed that bicuculline could prevent the acid stimulant action of muscimol only when the antagonist:agonist dose ratio ranged from 1:1 to 50:1 (Levine *et al.*, 1981; Del Tacca *et al.*, 1990).

Taking into account the mechanisms underlying the GABA-A-mediated effects on gastric secretory functions, previous studies suggested that central GABAergic pathways can modulate acid secretion through the activation or the inhibition of efferent vagal cholinergic neurones (Levine *et al.*, 1981; Andrews & Wood, 1986; Del Tacca *et al.*, 1990). Under our experimental conditions, atropine and pirenzepine were devoid of significant effects on both pepsinogen and acid secretion due to the deep inhibition of the vagal cholinergic tone that is commonly observed following anaesthesia with urethane (Maggi & Meli, 1986). Accordingly, the present findings, indicating that pirenzepine, a muscarinic receptor antagonist unable

to penetrate into the central nervous system (Kobayashi *et al.*, 1981), as well as atropine, could suppress both bicuculline- and muscimol-induced gastric hypersecretory effects, support the view that central GABA-A receptors can affect peptic or acid secretion through the involvement of pathways sensitive to the blockade of peripheral cholinergic activity. Similar results have been previously obtained following the subcutaneous administration of atropine to pylorus-ligated rats (Levine *et al.*, 1981). On the other hand, our data, showing that the stimulant actions elicited by bicuculline or muscimol were not affected by bilateral cervical vagotomy, suggest that these effects are mediated by extravagal pathways modulating the activity of the gastric cholinergic network. Somatostatin was shown to stimulate basal pepsinogen secretion in rats (Seefried, Schmidtler & Schwille, 1988), and the activation of central GABA-A receptors promotes an inhibition of somatostatin release (Gamse *et al.*, 1980). Accordingly, it might be speculated that the central injection of bicuculline or SR-95531 removes a tonic inhibition exerted by GABA-A receptors on the release of somatostatin or other neuropeptides endowed with stimulatory activity on pepsinogen secretion.

In conclusion, this study provides new evidence that central GABA-A receptors mediate a tonic inhibitory control on gastric pepsinogen secretion, while their phasic activation leads to an excitatory effect on acid output. In addition, central GABA-A receptors appear to affect the gastric secretory functions through extravagal mechanisms modulating the activity of peripheral cholinergic pathways.

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