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# Role of peripheral $GABA_B$ receptors in the regulation of pepsinogen secretion in anaesthetized rats

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**Abstract**

The purpose of the present study was to investigate the role played by $GABA_B$ receptors in the regulation of gastric basal pepsinogen secretion in anaesthetized rats. Following parenteral administration, the $GABA_B$ receptor agonists (−)-baclofen and 3-aminopropylphosphinic acid (3-APPA) caused a dose-dependent increase in basal pepsinogen secretion which was associated with a parallel increment in acid output. The gastric stimulant effects induced by both agonists were not affected by intracerebroventricular injection of the $GABA_B$ receptor antagonists 2-hydroxy-saclofen, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348) or phaclofen, whereas the excitatory actions were antagonized by intravenously administered 2-hydroxy-saclofen or CGP 35348, but not phaclofen. In addition, the (−)-baclofen-induced increases in both pepsinogen and acid output, were fully prevented by omeprazole or cimetidine, partly reduced by atropine and unaffected by pretreatment with capsaicin. When tested on rats undergoing bilateral cervical vagotomy, both (−)-baclofen and 3-APPA were still able to stimulate the basal pepsinogen and acid secretions, although at a lesser extent than in animals with intact vagus nerves. The stimulant actions elicited by (−)-baclofen in vagotomized rats were antagonized by 2-hydroxy-saclofen or CGP 35348, but not phaclofen. Moreover, these gastric excitatory effects were prevented by cimetidine or compound 48/80, while being unaffected by atropine. The present results show that peripheral $GABA_B$ receptors mediate an excitatory effect on gastric pepsinogen secretion which totally depends on an increase in acid output. It is also suggested that both vagal cholinergic and extravagal pathways, probably histaminergic in nature, take part in these $GABA_B$ receptor-mediated gastric stimulant actions.

*Keywords:* $GABA_B$ receptor; (−)-Baclofen; 3-Amino-propylphosphinic acid; Phaclofen; CGP 35348; 2-Hydroxy-saclofen; Gastric pepsinogen secretion; Gastric acid secretion

## 1. Introduction

Previous investigations have provided consistent evidence that the neurotransmitter γ-aminobutyric acid (GABA) plays a significant role in the regulation of gastric motility (Andrews et al., 1987; Wood et al., 1987) as well as of gastric acid secretion in different mammalian species, including man (Pugh et al., 1985; Andrews and Wood, 1986; Thirlby et al., 1988; Del Tacca et al., 1990). However, very scarce attention has been paid so far to the possible influence exerted by central or peripheral GABAergic pathways on the control of gastric pepsinogen secretion.

In a recent study, central injection of bicuculline to anaesthetized rats was associated with a significant increase in basal pepsinogen secretion without parallel changes in acid output, suggesting that central $GABA_A$ receptors are tonically activated to mediate an inhibitory control on peptic secretion (Natale et al., 1995). On the other hand, the $GABA_B$ receptor agonist baclofen markedly stimulated basal acid output after parenteral administration to various in vivo rat preparations (Goto and Debas, 1983; Andrews and Wood, 1986; Blandizzi et al., 1991), but the possibility that this drug could also affect the pepsinogen secretion was not investigated.

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Several aspects concerning the gastric hypersecretory effect evoked by parenterally administered baclofen remain also to be clarified. For instance, while central phaclofen-sensitive $GABA_B$ receptors mediate inhibitory effects of baclofen on acid secretion (Del Tacca et al., 1990), the gastric excitatory action exerted by this drug could not be antagonized by phaclofen (Blandizzi et al., 1991). In addition, it has been claimed that parenteral baclofen acts at central level to activate the parasympathetic outflow to the stomach (Goto and Debas, 1983; Andrews and Wood, 1986). However, findings obtained in subsequent studies appear to stand in contrast with this view, and they rather suggest that peripheral mechanisms might participate in the baclofen-induced gastric stimulant response (Thirlby et al., 1988; Blandizzi et al., 1991).

Overall, the present study was undertaken in order to: (1) investigate the effects of (−)-baclofen on basal pepsinogen secretion in the anaesthetized rat model; (2) characterize the profile of GABA receptors involved in the gastric stimulant action of baclofen by testing the effects of different agonists and antagonists; (3) gain more insight into the mechanisms underlying the gastric hypersecretory actions of baclofen.

## 2. Materials and methods

### 2.1. Animals and drug administration

The experiments were carried out on male Wistar rats, weighing about 200 g. They were fed standard laboratory chow and tap water ad libitum, and were not used for at least 1 week after their delivery to the laboratory. The animals were housed, six in 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.

In some animals, 3 or 4 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) given intraperitoneally (i.p.), as previously reported by Caulfield et al. (1983).

24 h before the experiments, the animals were maintained in single cages, provided with wire net bottoms in order to prevent coprophagy, and deprived of food. Free access to water ad libitum was allowed until 1 h 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 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.

### 2.2. Anaesthetized stomach-perfused rats

Continuous perfusion of the rat stomach in situ was carried out following the procedure developed by Ghosh and Schild (1958). The animals were anaesthetized with urethane (1.2 g/kg 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, 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. In order to ensure that the vagal trunks could not still favour the transmission of afferent or efferent nervous activity after cutting, both the proximal and distal end of each vagal trunk were mechanically damaged by crushing them with forceps (Natale et al., 1995).

Additional experiments were carried out in animals pretreated with the gastric proton pump blocker omeprazole (90 μmol/kg i.v.) 90 min before starting the collection of the gastric effluent fractions. The dose of omeprazole was selected because of its ability to acutely suppress both basal and stimulated gastric acid secretion in anaesthetized rats (Blandizzi et al., 1994).

A further set of experiments was designed to assess whether gastric capsaicin-sensitive sensory nerve fibres are involved in the gastric hypersecretory effect of (−)-baclofen. For this purpose, a group of animals underwent the administration of a total dose of 125 mg/kg capsaicin by subcutaneous (s.c.) route, as previously reported by Holzer et al. (1991). This dose of capsaicin was given over 2 days, with 25 mg/kg in the morning and 50 mg/kg in the late afternoon of the first day and 50 mg/kg on the second day. Capsaicin was dissolved (40 mmol/l) in a vehicle composed by 10% ethanol, 10% Tween 80 and 80% saline solution (v/v/v). All injections of capsaicin were performed under ether anaesthesia, and 10 days after this treatment the animals were used for the assessment of gastric acid secretion. 1 day before the experiments, the efficacy of the capsaicin treatment was checked by

instilling a drop of a capsaicin solution (0.1 mg/ml in saline solution) into one eye of each rat. Capsaicin-treated rats were expected not to react by wiping their eyes but, whenever an animal responded with wiping, the afflicted eye was immediately and extensively rinsed with water.

The polybasic compound 48/80 was repeatedly administered to a group of animals in order to induce a marked depletion of tissue histamine content. Treatment with compound 48/80 was carried out according to the 'short-term experiment' protocol proposed by Riley and West (1955), with minor modifications. Injections of compound 48/80 were given twice daily (9 a.m. and 5 p.m.) by i.p. route for 6 days, starting at 200 μg/rat per dose and continuing with 400, 600, 800, 1000 and 1000 μg/rat per dose, respectively. Experiments for the assessment of gastric secretion were performed after 15 h elapsed from the last admnistration of compound 48/80. Under similar experimental conditions, compound 48/80 was shown to induce a 93–97% reduction of normal histamine content in different rat tissues (Riley and West, 1955).

### 2.3. 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, 2 ml of 2.5% bovine haemoglobin were added to tubes containing 0.5 ml of 0.3 N HCl and 0.5 ml of gastric effluent. Samples were then incubated for 10 min at 37°C and the reaction was stopped by the 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. The results were compared to a standard curve generated in an identical manner, using known amounts of porcine pepsin (1 μg = 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) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH. Data were expressed as μEq $H^+$/15 min.

### 2.4. Drugs

The following drugs and reagents were used: (−)-baclofen and 3-aminopropyl(diethoxymethyl)phosphinic acid [CGP 35348] (both kindly provided by Ciba-Geigy, Saronno, Italy); 3-aminopropylphosphinic acid (3-APPA), phaclofen, and 2-hydroxy-saclofen (Tocris Neuramin, Bristol, England); capsaicin, compound 48/80, urethane (ethyl carbamate), crystallized

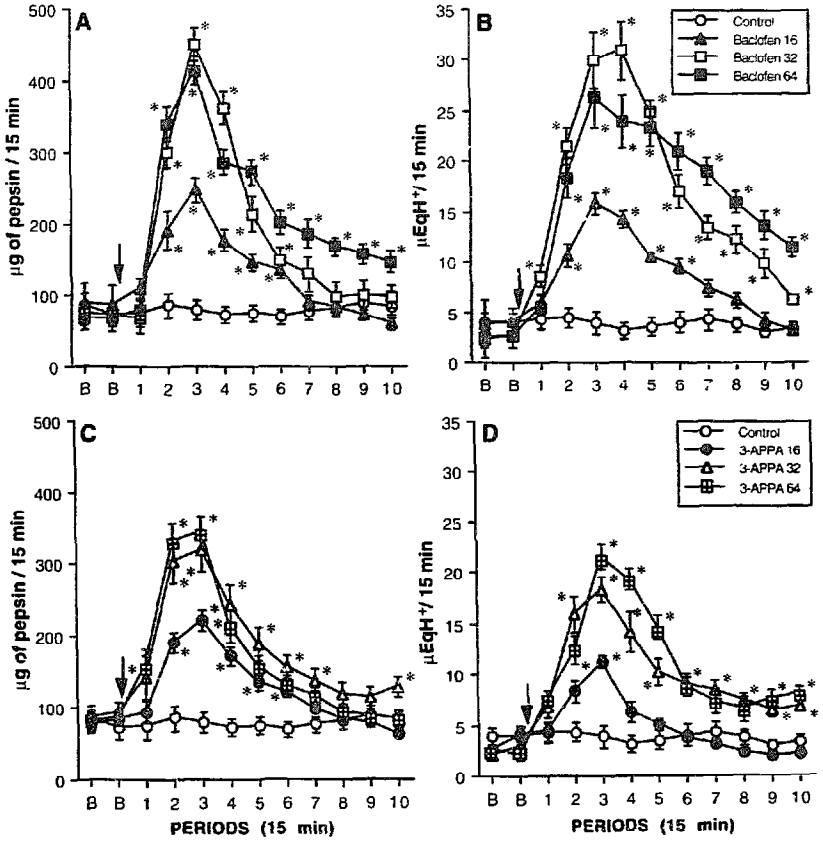


Fig. 1. Stomach-lumen perfused rats with intact vagus nerves. Effects of baclofen (16, 32 and 64 μmol/kg i.v.) or 3-APPA (16, 32 and 64 μmol/kg i.v.) on basal pepsinogen (A and C) and acid (B and D) secretion. Each point represents the mean value obtained from eight to ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of agonist administration. B = basal value. $^*P < 0.05$: significant difference from control values.

porcine pepsin, and lyophilized bovine haemoglobin (Sigma, St. Louis, MO, USA); cimetidine (Italfarmaco, Milan, Italy); atropine sulfate (BDH Chemicals, Poole, England); omeprazole (Malesci, Florence, Italy); pentobarbital sodium (Clin-Midy, Paris, France). Other reagents were of analytical grade.

### 2.5. Statistics

Results are given as means ± S.E.M. The significance of differences was evaluated by one way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, and $P$ values lower than 0.05 were considered significant; '$n$' indicates the number of experiments.

## 3. Results

### 3.1. Effects of $GABA_B$ receptor agonists on animals with intact vagus nerves

In control animals, receiving drug vehicle ($n = 8$), basal gastric pepsinogen and acid secretions, assessed after 30-min stabilization, accounted for 87.1 ± 14.4 μg of pepsin/15 min and 4.1 ± 0.9 μEq $H^+$/15 min respectively, and these values remained at a steady level up to the end of the experimental period (150 min).

Under these conditions, (−)-baclofen (16, 32 and 64 μmol/kg i.v.; $n = 10$ for each dose) caused a dose-dependent and parallel increase in both pepsinogen and acid secretions, the maximal stimulatory effects occurring at the dose of 32 μmol/kg (Fig. 1A,B). The excitatory responses exerted by (−)-baclofen on both peptic and acid outputs, were mimicked by 3-aminopropylphosphinic acid (3-APPA; 16, 32 and 64 μmol/kg i.v.; $n = 10$ for each dose) which induced a maximal increment of gastric secretions at the dose of 32 μmol/kg (Fig. 1C,D). However, the hypersecretory effects elicited by 3-APPA (peak increments at 32 μmol/kg: 317.6 ± 28.1 μg of pepsin/15 min and 18.3 ± 2.0 μEq $H^+$/15 min) were significantly lower than those obtained in the presence of (−)-baclofen (peak increments at 32 μmol/kg: 451.8 ± 23.2 μg of pepsin and 31.0 ± 3.4 μEq $H^+$/15 min; $P < 0.05$ versus 3-APPA-induced peptic and acid secretions, respectively).

### 3.2. Effects of $GABA_B$ receptor antagonists on animals with intact vagus nerves

The intracerebroventricular administration of phaclofen (128 and 256 nmol/rat; $n = 6$ for each dose), CGP 35348 (64 nmol/rat; $n = 6$), or 2-hydroxy-saclofen (64 nmol/rat; $n = 6$) did not significantly affect basal gastric secretions as well as the hypersecretory effects induced by (−)-baclofen 32 μmol/kg i.v. ($n = 6$ for each antagonist; not shown). In addition, the intravenous injection of phaclofen (128 and 256 μmol/kg; $n = 6$ for each dose), CGP 35348 (64 μmol/kg; $n = 6$) or 2-hydroxy-saclofen (64 μmol/kg; $n = 6$) did not modify the basal peptic and acid outputs (not shown). However, the stimulant actions exerted by (−)-baclofen (32 μmol/kg i.v.) on both peptic and acid secretions were not affected by phaclofen (128 and 256 μmol/kg i.v.; $n = 10$ for each dose), but they were partly antagonized by CGP 35348 (64 μmol/kg i.v.; $n = 10$), and completely prevented by 2-hydroxy-saclofen (64 μmol/kg i.v.; $n = 10$) (Fig. 2A,B). Similar results were obtained also when testing the effects of intravenously administered phaclofen (128 and 256 μmol/kg i.v.; $n = 10$ for each dose), CGP 35348 (64 μmol/kg i.v.; $n = 10$) or 2-hydroxy-saclofen (64 μmol/kg i.v.; $n = 10$) on both peptic and acid secretions evoked by 3-APPA (32 μmol/kg i.v.) (Fig. 2C,D).

### 3.3. Effects of (−)-baclofen on animals with intact vagus nerves pretreated with gastric antisecretory drugs or capsaicin

In animals with intact vagus nerves, the gastric stimulant actions elicited by intravenous (−)-baclofen (32 μmol/kg i.v.) were partly reduced by pretreatment with atropine (1 μmol/kg i.v.; $n = 8$), while they were completely prevented by cimetidine (10 μmol/kg i.v.; $n = 8$) or omeprazole (90 μmol/kg i.v.; $n = 8$) (Fig. 3). Atropine could not fully antagonize peptic or acid hypersecretion elicited by (−)-baclofen even when tested at the doses of 2 or 4 μmol/kg i.v. ($n = 6$ for each dose). Indeed, in animals pretreated with atropine 4 μmol/kg the (−)-baclofen-induced peak increments in pepsinogen and acid outputs accounted for 266.5 ± 18.3 μg of pepsin/15 min and 13.4 ± 1.3 μEq $H^+$/15 min, respectively ($P < 0.05$ versus basal secretory values obtained in control animals). In addition, the functional ablation of capsaicin-sensitive sensory neurons induced by pretreatment with capsaicin ($n = 10$) failed to significantly modify the excitatory effects exerted by (−)-baclofen (32 μmol/kg i.v.) on both pepsinogen and acid secretions (not shown).

### 3.4. Effects of $GABA_B$ receptor agonists and antagonists on animals with bilateral cervical vagotomy

When administered to acutely vagotomized rats, both (−)-baclofen (32 μmol/kg i.v.; $n = 10$) and 3-APPA (32 μmol/kg i.v.; $n = 10$) caused a significant increase in basal peptic and acid secretions (Fig. 4). For both drugs the gastric stimulant responses detected in vagotomized rats were significantly lower than those obtained with the same doses in animals with intact vagus nerves (Fig. 4).

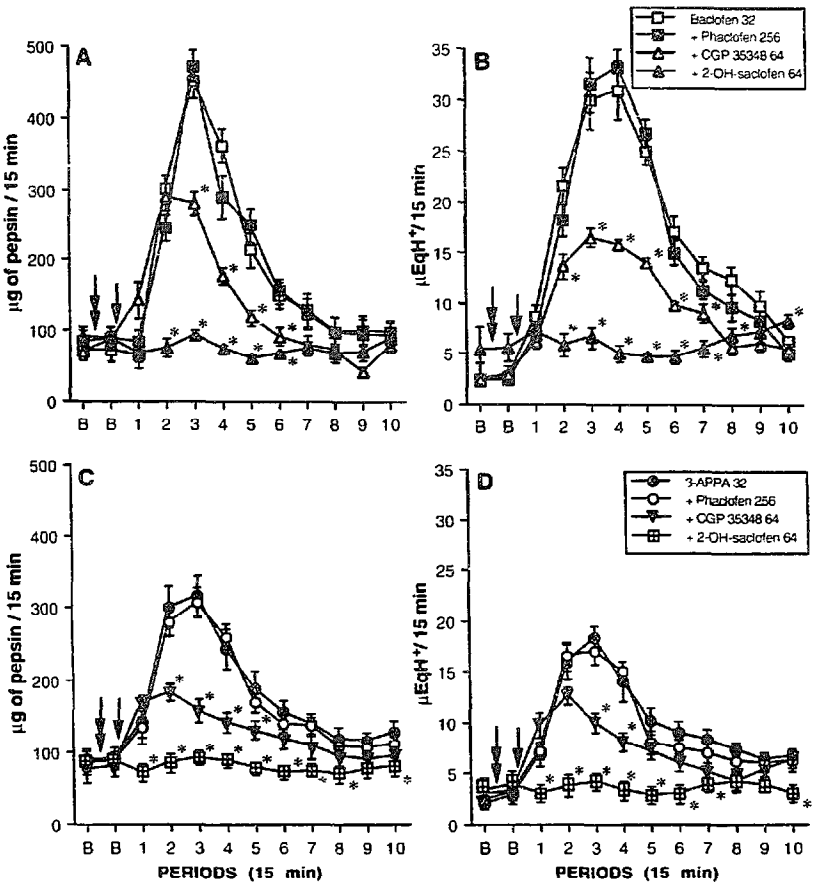


Fig. 2. Stomach-lumen perfused rats with intact vagus nerves. Effects of baclofen (32 μmol/kg i.v.) or 3-APPA (32 μmol/kg i.v.), administered either alone or in the presence of phaclofen (256 μmol/kg i.v.), CGP 35348 (64 μmol/kg i.v.), or 2-hydroxy-saclofen (64 μmol/kg i.v.) on basal pepsinogen (A and C) and acid (B and D) secretion. Each point represents the mean value obtained from ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of agonist administration; the double arrow indicates the time of antagonist administration. B = basal value. $^{*}P < 0.05$: significant difference from either baclofen or 3-APPA alone.

Under the same experimental conditions, the hypersecretory effects exerted by (−)-baclofen (32 μmol/kg i.v.) on both pepsinogen and acid outputs were not significantly affected by pretreatment with phaclofen (128 and 256 μmol/kg i.v.; $n = 10$ for each dose), whereas they were partly antagonized by CGP 35348

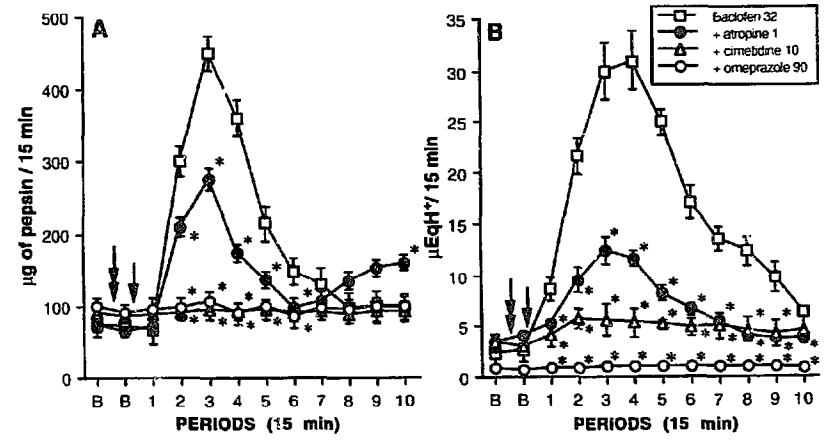


Fig. 3. Stomach-lumen perfused rats with intact vagus nerves. Effects of baclofen (32 μmol/kg i.v.), administered either alone or after pretreatment with atropine (1 μmol/kg i.v.), cimetidine (10 μmol/kg i.v.), or omeprazole (90 μmol/kg i.v.), on basal pepsinogen (A) and acid (B) secretion. Each point represents the mean value obtained from eight to ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of baclofen administration; the double arrow indicates the time of atropine or cimetidine administration. Omeprazole was administered 90 min before the collection of basal effluent samples. B = basal value. $^{*}P < 0.05$: significant difference from baclofen alone.

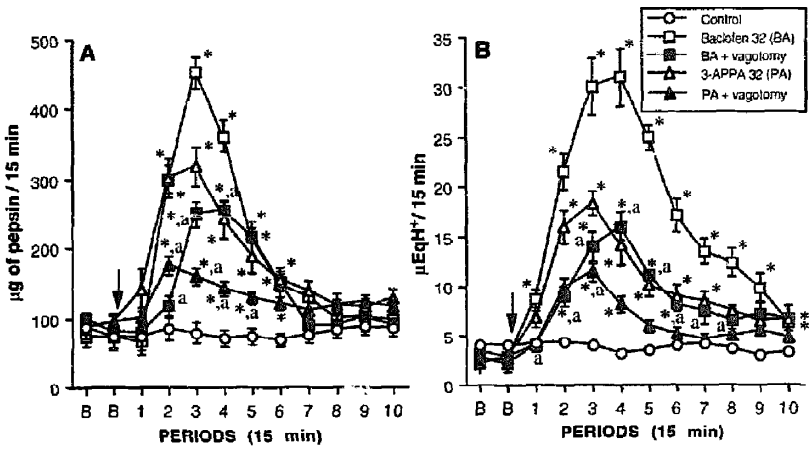


Fig. 4. Stomach-lumen perfused rats. Effects of baclofen (32 μmol/kg i.v.) or 3-APPA (32 μmol/kg i.v.), both administered to animals with intact vagus nerves or undergoing bilateral cervical vagotomy, on basal pepsinogen (A) and acid (B) secretion. Each point represents the mean value obtained from eight to ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of agonist administration. Bilateral cervical vagotomy was performed about 30 min before the collection of basal effluent samples. B = basal value. $^{*}P < 0.05$: significant difference from control values; $^{a}P < 0.05$: significant difference from either baclofen or 3-APPA administered to animals with intact vagus nerves.

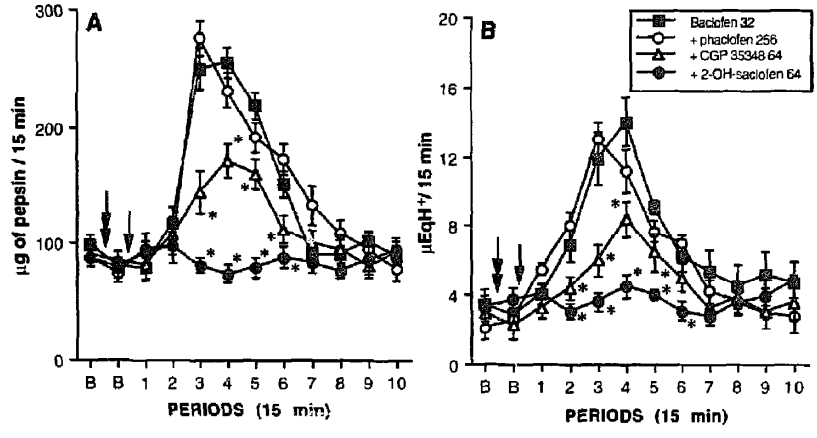


Fig. 5. Stomach-lumen perfused rats with bilateral cervical vagotomy. Effects of baclofen (32 μmol/kg i.v.), administered either alone or in the presence of phaclofen (256 μmol/kg i.v.), CGP 35348 (64 μmol/kg i.v.), or 2-hydroxy-saclofen (64 μmol/kg i.v.) on basal pepsinogen (A) and acid (B) secretion. Each point represents the mean value obtained from ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of baclofen administration; the double arrow indicates the time of antagonist administration. B = basal value. $^{*}P < 0.05$: significant difference from baclofen alone.

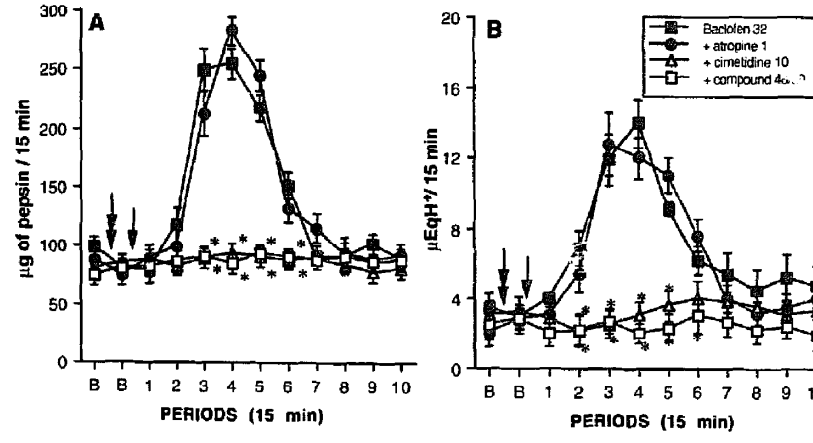


Fig. 6. Stomach-lumen perfused rats with bilateral cervical vagotomy. Effects of baclofen (32 μmol/kg i.v.), administered either alone or after pretreatment with atropine (1 μmol/kg i.v.), cimetidine (10 μmol/kg i.v.), or compound 48/80, on basal pepsinogen (A) and acid (B) secretion. Each point represents the mean value obtained from eight to ten animals ± S.E.M. (vertical lines). The single arrow indicates the time of baclofen administration; the double arrow indicates the time of atropine or cimetidine administration. The compound 48/80 was administered at increasing doses (from 400 to 2000 μg/day per rat) during 6 days preceeding the beginning of the experiment. B = basal value. $^{*}P < 0.05$: significant difference from baclofen alone.

(64 μmol/kg i.v.; $n = 10$), and completely prevented by 2-hydroxy-saclofen (64 μmol/kg i.v.; $n = 10$) (Fig. 5).

### 3.5. Effects of (−)-baclofen on animals with bilateral cervical vagotomy pretreated with gastric antisecretory drugs or compound 48/80

The gastric hypersecretory actions evoked by (−)-baclofen 32 μmol/kg i.v. on rats undergoing bilateral cervical vagotomy were not significantly modified by pretreatment with atropine (1, 2 and 4 μmol/kg i.v.; $n = 6$–8 for each dose) (Fig. 6). In particular, in animals pretreated with atropine 4 μmol/kg ($n = 6$), the (−)-baclofen-induced peak increments in pepsinogen and acid outputs accounted for 263.4 ± 13.3 μg of pepsin/15 min and 13.3 ± 1.9 μEq $H^+$/15 min, respectively, these secretory values being not significantly different from those obtained in the presence of (−)-baclofen alone (254.7 ± 12.9 μg of pepsin/15 min and 14.1 ± 1.4 μEq $H^+$/15 min, respectively).

Furthermore, after pretreatment of vagotomized rats with compound 48/80 in order to obtain a marked depletion of histamine tissue content, (−)-baclofen 32 μmol/kg i.v. ($n = 8$) failed to modify both basal pepsinogen and acid secretions (Fig. 6).

## 4. Discussion

Previous studies have reported that the parenteral administration of baclofen causes a marked increase in gastric acid secretion in humans, dogs and rats (Pugh et al., 1985; Andrews and Wood, 1986; Thirlby et al., 1988; Blandizzi et al., 1991), however the role played by $GABA_B$ receptors in this effect has not been established. In the present investigation, the use of different $GABA_B$ receptor agonists and antagonists allowed us to obtain more consistent evidence for the involvement of these receptors in the control of acid secretion as well as to extend this notion to the output of gastric pepsinogen output.

The experiments performed on animals with intact vagus nerves revealed that the gastric hypersecretory effects exerted by (−)-baclofen could be reproduced also by 3-APPA, a selective $GABA_B$ receptor agonist (Hills et al., 1989). However, although 3-APPA has been shown to exhibit about 10-fold higher affinity than baclofen for $GABA_B$ receptor sites in binding studies (Bowery, 1993), in our hands the gastric stimulant responses elicited by 3-APPA were significantly lower than those obtained with (−)-baclofen at equimolar doses. In this regard, it is noteworthy that, in contrast with the findings from binding experiments, significant discrepancies have been reported in functional assays dealing with the respective potency and efficacy of baclofen and 3-APPA (Hills et al., 1989; Seabrook et al., 1990). In particular, electrophysiological experiments performed on rat brain slices indicated that, although both agonists were roughly equipotent in affecting neuronal electrical responses, 3-APPA displayed a maximal effect of about 70–80% that of baclofen, suggesting that 3-APPA has a lower intrinsic efficacy than baclofen and may act as a partial agonist on $GABA_B$ receptors. Accordingly, these pharmacological characteristics might well account for the different efficacy exhibited by (−)-baclofen and 3-APPA in stimulating the gastric secretions in the present study.

Irrespectively of their different efficacy, both (−)-baclofen and 3-APPA evoked an increase in pepsinogen secretion that paralleled the increment observed in acid output, thus suggesting the possible activation of common mechanisms. Indeed, pepsinogen secretion generally increases in several gastric preparations as an indirect response to agents that primarily stimulate acid secretion (Hersey, 1987). This parallelism appears to depend mainly on the ability of gastric acidity to enhance the pepsinogen output through the activation of local nervous reflexes (Johnson, 1972; Smith and Torres, 1990). As a consequence, in the attempt to discriminate between direct or indirect (namely, acid-dependent) stimulation of peptic secretion, the benzimidazole derivative omeprazole has been previously used in both in vitro (Basson et al., 1988) and in vivo preparations (Blandizzi et al., 1994), due to the unique property of this drug of suppressing acid secretion through a blockade of the gastric $H^+$:$K^+$-ATPase, without affecting any receptor or transduction pathway at cellular level (Clissold and Campoli-Richards, 1986). On these bases, the present data, showing that both acid and peptic hypersecretory effects induced by (−)-baclofen were completely prevented by pretreatment with omeprazole, suggest that the pepsigogue action of (−)-baclofen represents an epiphenomenon depending totally on the increase in acid output. By contrast, central $GABA_A$ receptors appear to play a differential role in the regulation of gastric pepsinogen and acid secretion, respectively (Natale et al., 1995).

The present results, showing that in animals with either intact vagus nerves or bilateral vagotomy the gastric excitatory effects evoked by (−)-baclofen or 3-APPA displayed differential sensitivities to parenterally administered $GABA_B$ receptor antagonists, deserve further considerations. On the basis of data obtained by functional experiments concerning neurotransmitter release, the existence of distinct $GABA_B$ receptor subtypes has been suggested (Bonanno and Raiteri, 1993a; Bowery, 1993), and a systematic classification of these receptors has been proposed (Bonanno and Raiteri, 1993b). According to this classification scheme, the $GABA_B$ receptors involved in the present gastric hypersecretory effects of (−)-baclofen or 3-APPA might be tentatively assigned to the $GABA_{B1\beta}$

receptor subtype (namely, phaclofen-insensitive and CGP 35348-sensitive) (Bonanno and Raiteri, 1993b). However, a conclusive characterization of $GABA_B$ receptor subtypes needs further contributions from both binding and molecular biology studies. Moreover, caution is needed when trying to interpret the results of in vivo functional experiments in terms of differential receptor sensitivity to different ligands. In addition, the currently proposed classification of $GABA_B$ receptor subtypes has not encountered unanimous recognition (Waldmeier et al., 1994). Overall, the fact that the gastric stimulant actions of the $GABA_B$ receptor agonists were not affected by phaclofen, partly prevented by CGP 35348 and fully antagonized by 2-hydroxy-saclofen might be simply consistent with the rank order of affinity obtained for these antagonists in radioligand binding studies: 2-hydroxy-saclofen > CGP 35348 > > phaclofen (Bowery, 1993). On the other hand, the marked antagonistic action exerted by 2-hydroxy-saclofen in the present study does not appear attributable to a putative anticholinergic activity, since radioligand experiments showed that 2-hydroxy-saclofen does not possess affinity for muscarinic receptor sites within the micromolar range (Al-Dahan et al., 1990).

Conflicting findings have been reported concerning the location of receptor sites and pathways implicated in the gastric hypersecretory action of (−)-baclofen. In previous investigations, the observation that the baclofen-induced acid stimulant action was prevented by bilateral vagotomy or atropine led to the suggestion that parenteral baclofen, being a lipophilic derivative of GABA, readily crosses the blood-brain barrier, thus activating a central parasympathetic outflow to the stomach (Goto and Debas, 1983; Andrews and Wood, 1986). In accordance with this hypothesis, further studies showed that centrally applied baclofen significantly stimulated gastric motility in the rat, this effect being counteracted by either vagotomy or pretreatment with atropine (Wood et al., 1987). Nevertheless, several lines of evidence do not support the view that the gastric hypersecretory action of parenteral baclofen is entirely mediated by a direct interaction with central sites. First, the central injection of baclofen failed to reproduce the marked gastric stimulation observed after its parenteral administration, rather causing a significant inhibition or at most a slight increment of acid output (Del Tacca et al., 1990; Blandizzi et al., 1991). Second, parenteral baclofen was able to enhance gastric secretion from vagally denervated Heidenhain pouch dogs (Thirlby et al., 1988) as well as from conscious rats whose vagal activity was already markedly stimulated by pylorus ligation (Blandizzi et al., 1991). Third, some of the $GABA_B$ receptor antagonists tested in the present study were able to prevent the (−)-baclofen- and 3-APPA-induced gastric hypersecretion only when administered by intravenous, but not by intracerebroventricular route. Fourth, the gastric stimulant actions exerted by both (−)-baclofen and 3-APPA could be prevented only in part by bilateral cervical vagotomy or atropine. Taken together, all these data are consistent with the hypothesis that the $GABA_B$ receptors mediating excitatory effects on gastric secretions are located at peripheral level and that, once activated, they may recruit at least two different mechanisms: (1) a vago-vagal reflex which causes a centrally driven cholinergic output to the stomach, accounting for the sensitivity of the stimulant actions of $GABA_B$ agonists to vagotomy or atropine; (2) a local extravagal pathway, not cholinergic in nature, accounting for the vagotomy- and atropine-resistant excitatory effects of (−)-baclofen and 3-APPA. On the other hand, since the central administration of baclofen caused a consistent stimulation of gastric motility (Wood et al., 1987) as well as a weak increase in acid secretion (Del Tacca et al., 1990), a central site of action appears also to be somehow involved in the gastric stimulant actions of this drug. Therefore, the currently available evidence deriving from gastric motility studies favours a predominantly central site of action for baclofen, whereas with regard to the secretory studies the weight of evidence is strongly in favour of a larger contribution by peripheral $GABA_B$ receptors.

The view that the (−)-baclofen-induced gastric hypersecretion appears to be generated at peripheral level, prompted us to investigate the possible involvement of capsaicin-sensitive sensory neurones in this stimulant effect. Indeed, capsaicin-sensitive afferent nerves have been described in the gastric mucosa (Green and Dockray, 1988) as well as in the vagus nerve (Sharkey et al., 1991), and they were significantly implicated in the regulation of some gastric functions, including acid secretion (Maggi and Meli, 1988). In particular, the activation of mucosal afferent nerves by acute intragastric application of capsaicin decreased acid secretion in rats and dogs (Lippe et al., 1989; Soldani et al., 1992). Moreover, both the ablation of capsaicin-sensitive sensory fibers and bilateral cervical vagotomy were shown to inhibit the acid hypersecretion evoked by gastric distension, a response which involves the activation of a centrally driven vago-vagal reflex (Raybould and Taché, 1989). However, in spite of these findings, the present data, showing that systemic pretreatment with capsaicin did not affect acid and peptic outputs evoked by (−)-baclofen, suggest that capsaicin-sensitive sensory nerves do not participate in the vagus-dependent component of the gastric secretory responses mediated by peripheral $GABA_B$ receptors.

As far as the vagus nerve-independent component of (−)-baclofen-induced gastric hypersecretion is concerned, it is interesting to note that this response,

although resistant to atropine, was completely prevented by pretreatment with the histamine $H_2$ receptor antagonist cimetidine as well as with compound 48/80, a potent histamine-depletant (Riley and West, 1955). In this regard, data supporting the existence of mutual interactions between GABAergic and histaminergic pathways in the central nervous system have been reported (Crawford and Young, 1988; Waskiewicz and Rafalowska, 1992). At peripheral level, several lines of evidence indicate that some secretory cells of the gastric mucosa contain GABA-like immunoreactivity and possess specific mechanisms for GABA uptake (Erdo and Wolff, 1988; Jessen et al., 1988). In addition, GABA co-localizes with gastrin in G cells of rat antral mucosa (Davanger et al., 1994), suggesting that this neurotransmitter may act as a gastric hormone (Jessen et al., 1988). According to these findings, our data support the view that the activation of peripheral $GABA_B$ receptors might stimulate gastric secretion, at least in part, through an involvement of gastric histaminergic pathways. Probably, the most important evidence in support of this view is represented by our experiments performed in the presence of cimetidine. However, since compound 48/80 lacks specific actions on digestive tissues, the actual implication of gastric histamine in the stimulant actions exerted by baclofen on rat stomach requires more extensive investigation. On the other hand, a direct localization of $GABA_B$ receptors on parietal cells does not appear conceivable, since baclofen was found to be devoid of effects on different in vitro stomach preparations (Tsai et al., 1987; Blandizzi et al., 1991).

In conclusion, the present study provides evidence that the systemic administration of $GABA_B$ receptor agonists stimulates the gastric pepsinogen secretion indirectly through an increase in acid output. In addition, it is suggested that both vagal cholinergic and extravagal pathways, possibly histaminergic in nature, take part in these gastric stimulant effects via the activation of peripheral $GABA_B$ receptors.

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