treated for rheumatic arthritis [12]. Furthermore, it is impossible to exclude the likelihood of harmful effects other than mortality which we were not able to detect, as indicated for example, by temporary weight loss in surviving animals. Thus, there is a possibility of unnoticed harmful impact of the interaction of salicylate and noise in humans.

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GABA-A-mediated gastrin release induced by baclofen in the isolated vascularly perfused rat stomach

M. Del Tacca, C. Blandizzi, M. C. Bernardini, [a] F. Martelli and [a] A. Della Longa

Institute of Medical Pharmacology, Via Roma 55, and [a] Institute of Physiology and Biochemistry, Veterinary Medical School, Via Piagge 2, University of Pisa, I-56100 Pisa (Italy)

Summary. In order to investigate the role of peripheral GABA-B receptors, the effects of the putative GABA-B agonist baclofen on immunoreactive gastrin release from an isolated vascularly perfused rat stomach preparation were examined. The vascular infusion of baclofen at graded concentrations induced a dose-dependent increase in gastrin release; this was unaffected by the GABA-B antagonist delta-aminovaleric acid, but was fully prevented by the selective GABA-A antagonist bicuculline as well as by atropine or tetrodotoxin. These results suggest that the stimulant effects of baclofen are mediated by nervous cholinergic structures associated with GABA-A receptors, and indicate that this GABA-B agonist must be regarded as a partial agonist of peripheral GABA-A receptors.
Key words. Gastrin release; rat isolated stomach; baclofen; GABA-A receptors.

Several lines of evidence indicate that GABA-A receptors participate in the regulation of gastric acid secretion. Experiments in vivo in anesthetized rats have demonstrated that activation of central GABA-A receptors is associated with a marked dose-dependent and bicuculline-sensitive increase in acid secretion [1,2].
Experiments in vitro on isolated guinea pig stomach have shown that the GABA-A receptor agonist muscimol induces a dose-dependent enhancement of acid secretion which is antagonized by bicuculline, but the putative GABA-B receptor agonist baclofen does not. This indicates that peripheral GABA-A, but not GABA-B receptors, participate in the regulation of acid secretion [3]. Moreover, in rat isolated gastric antral mucosal fragments, GABA produced a dose-dependent bicuculine- and atropine-sensitive stimulation of gastrin release, probably mediated by the activation of antral cholinergic neurons associated with GABA-A receptors [4].
Whether or not peripheral GABA-B receptors are involved in the regulation of gastrin release remains unknown. The purpose of the present study was to investigate the effects of baclofen on gastrin release from the isolated vascularly perfused rat stomach.

Methods

Experiments were carried out on male Sprague-Dawley rats weighing 200–220 g, 24 h fasted, but with free access to water.
Isolated vascularly perfused rat stomach. The isolation of the stomach was performed using the technique described by Saffouri et al. [5] and Martindale et al. [6], with minor modifications. The animals were anesthetized with

urethane (1 g/kg injected i.p.) and the abdomen was incised by means of a xipho-pubic cut. Then the pancreas, spleen and small bowel were removed after ligation of their vascular supply. The abdominal aorta was sectioned after ligation, just proximally and distally to the origin of the celiac axis. The portal vein was transected on the hepatic side after ligation.

The stomach was removed and placed in a humidity-saturated perfusion chamber, maintained at a temperature of 37 °C. The abdominal aorta, portal vein, esophagus and duodenum were cannulated. The aorta was perfused at a rate of 2 ml/min with Krebs-bicarbonate solution (NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHPO_4$ 1.2 and $NaHCO_3$ 22.0 mmol/l) (pH 7.4) containing 0.2% bovine serum albumin, 4% dextrane (mw 70,000) and 4.5 mM glucose. The perfusate was oxygenated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37 °C. The drugs under study were administered by continuous infusion through the aorta.

The gastric lumen was also perfused via the esophagus at a rate of 2 ml/min with saline solution (NaCl 0.9%), oxygenated with 100% O_2, and maintained at a temperature of 37 °C.

Under basal conditions the preparation exhibited stable baseline gastrin secretion over a period of 90 min. However, after examination of the profile of the concentration-response curves of the drugs under investigation, the period of 35 min for the duration of each experiment was selected, and employed throughout the study.

After a 30-min equilibration period the portal vein effluent was collected in ice-chilled containers at 5-min intervals for 35 min. All samples were stored at − 20 °C until the assay was performed.

Gastrin assay. The gastrin concentration was determined by means of radioimmunoassay, following the procedure previously reported[7]. Briefly, 100 µl of each sample was incubated for 18 h at room temperature with 100 µl of human ^{125}I-labelled gastrin and 100 µl of rabbit gastrin antiserum. The immunocomplexes were separated using 1 ml of 20% PEG (mw 6000) plus 0.1% Tween 20 solution. Samples were vortexed and centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant was aspirated and the pellet gamma radioactivity was counted for 1 min. The detection limit was 10 pg/ml. The mean recovery rate was 92 ± 6%.

Drugs

The following drugs were used: (±)baclofen (Ciba-Geigy S.p.A., Saronno, Varese, Italy), delta-aminovaleric acid, (−)bicuculline methiodide, bethanechol hydrochloride, atropine sulphate and tetrodotoxin (Sigma Chemicals Co., St. Louis, MO, USA).

Statistical analysis

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

Results

Over the 35-min observation period, basal concentrations of gastrin in the portal vein effluent ranged from 36 to 44 pg/ml (n = 8).

Baclofen (3, 10 and 30 µg/ml; n = 5 for each dose) produced a dose-dependent increase in gastrin release into the venous perfusate, the maximal effect occurring after a dose of 10 µg/ml (fig. 1). Delta-aminovaleric acid (DAVA) in doses up to 30 µg/ml (n = 5) did not affect the stimulant effect of baclofen (10 µg/ml), whereas higher doses gave erratic results. Bicuculline (1 to 10 µg/ml; n = 5 for each dose) fully prevented the stimulant effect of baclofen (fig. 2). Atropine (0.5 µg/ml; n = 5) or tetrodotoxin (1 µg/ml; n = 5) completely blocked the gastrin release evoked by baclofen (fig. 3).

Under the same basal conditions, bethanechol (0.1–1 µg/ml; n = 3 for each dose) caused a dose-dependent increase in gastrin release; the maximal increase (140 ± 11.2 pg/ml) was obtained with 1 µg/ml at the 20th min of the infusion. Submaximal doses of bethanechol (0.2 µg/ml) potentiated (+ 30%) submaximal (3 µg/ml) responses to baclofen (n = 4). The stimulant effects of bethanechol (1 µg/ml) were unaffected by bicuculline (10 µg/ml; n = 5), but were fully prevented by atropine (0.5 µg/ml; n = 5).

Discussion

The present results provide the first evidence indicating that the putative GABA-B receptor agonist baclofen

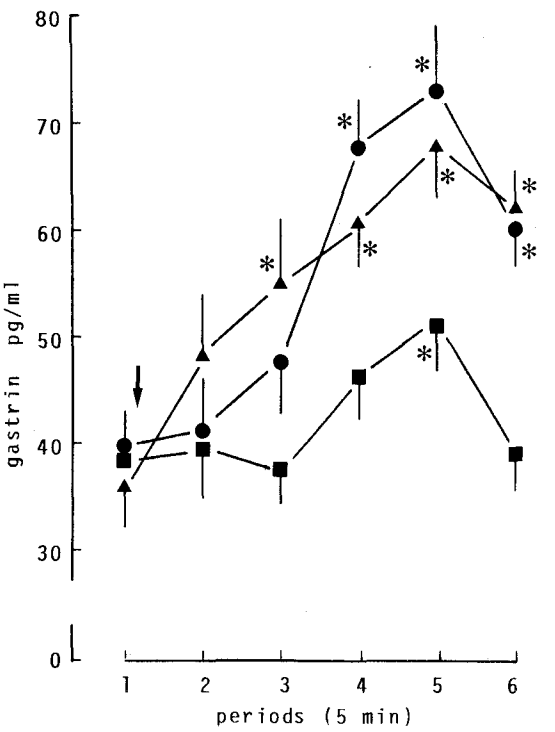

Figure 1. Effects of baclofen 3 (■), 10 (●), and 30 (▲) µg/ml on gastrin release (pg/ml). Each point represents the mean value of 5 experiments ± SE (vertical lines). The arrow indicates drug administration. Significant difference from control values (time 0): *p < 0.05.

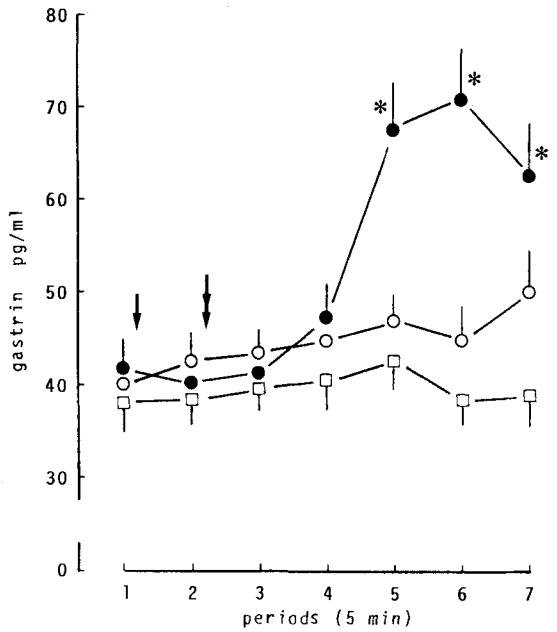

Figure 2. Effects of baclofen 10 μg/ml (●), bicuculline 10 μg/ml (□), and bicuculline plus baclofen (○) on gastrin release (pg/ml). Each point represents the mean value of 5 experiments ± SE (vertical lines). The single arrow indicates bicuculline administration; the double arrow indicates baclofen administration. Significant difference from control values (time 0): *$p < 0.05$.

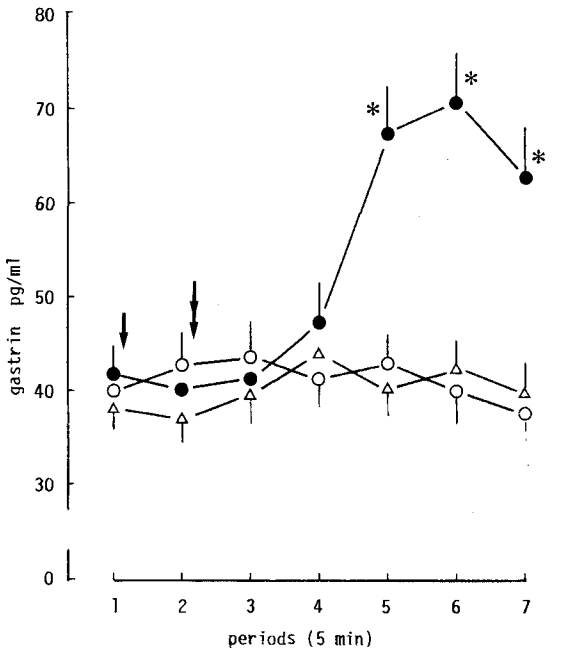

Figure 3. Effects of baclofen 10 μg/ml (●), atropine 0.5 μg/ml plus baclofen (○), and tetrodotoxin 1 μg/ml plus baclofen (△) on gastrin release (pg/ml). Each point represents the mean value of 5 experiments ± SE (vertical lines). The single arrow indicates atropine or tetrodotoxin administration; the double arrow indicates baclofen administration. Significant difference from control values (time 0): *$p < 0.05$.

stimulates gastrin release from the isolated vascularly perfused rat stomach. On the basis of the results obtained with tetrodotoxin and atropine, this effect appears to be mediated through nervous cholinergic structures. Moreover, the importance of cholinergic participation is further supported by the present bethanechol-mediated effects on gastrin release.

The selective blockade by bicuculline indicates that baclofen activates, at least in part, GABA-A receptors associated with cholinergic neurons. Furthermore, the lack of any influence of DAVA on baclofen-evoked gastrin release suggests that peripheral GABA-B receptors do not contribute to the stimulant effect of baclofen, although this result should be regarded as inconclusive since DAVA is not a potent antagonist of GABA-B receptors and possesses a partial agonist activity on GABA-A receptors[8]. A similar GABA-A mediated excitatory effect of baclofen has previously been observed on rat acid secretion[2], which suggests that the activity of the drug is not exclusively confined to GABA-B receptors. In support of GABA-A-mediated release of gastrin, Harty and Franklin[4] found that GABA induced a bicuculline-sensitive activation of cholinergic pathways allowing gastrin release from rat antral mucosal fragments. Additional indirect evidence for baclofen-induced gastrin release comes from the observations of Tsai et al.[3], who found that the gastrin receptor antagonist proglumide partially inhibited acid hypersecretion induced by the activation of GABA-A receptors in the isolated guinea pig stomach, which indicates that gastrin is partially involved in the acid secretory responses evoked by the activation of the GABA-A receptors. The lack of any hypersecretory effect of baclofen in the experiments of Tsai et al.[3] might be due to their experimental conditions, including the animal species and the doses employed.

Although the effects of baclofen described here appear to be indirect, mediated through the stimulation of cholinergic neurons, it remains to be determined whether baclofen may activate G cells directly.

Finally, the overall results indicate that baclofen behaves as a partial agonist of peripheral GABA-A receptors.

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