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Short communication

The effect of the $GABA_B$ receptor agonist baclofen on liquid and solid gastric emptying in mice

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Abstract

The effect of the $GABA_B$ receptor agonist baclofen, a potential treatment for gastroesophageal reflux, on gastric emptying has not been determined. The effect of 1–4 mg/kg baclofen on liquid and solid gastric emptying in mice was evaluated by noninvasive [^{13}C] breath tests. Baclofen accelerated gastric emptying of solids but delayed emptying of liquid, suggesting that it may have differential effects on proximal and distal stomach emptying.

Keywords: Baclofen; Breath test; $GABA_B$ receptor; Gastric emptying; (Mouse)

1. Introduction

The $GABA_B$ receptor agonist baclofen reduces the triggering of transient lower oesophageal sphincter relaxation and this, in turn, leads to a reduction in gastroesophageal reflux (Blackshaw et al., 1999; Lehmann et al., 1999; Lidums et al., 2000; Zhang et al., 2002). Baclofen has also been shown in rats, ferrets and mice to have a number of effects on gastrointestinal motility, including an augmentation of basal lower esophageal sphincter and gastric tone (Lidums et al., 2000; Monroe and Hornby, 2002), as well as an increase in the frequency and amplitude of gastric and duodenal contractions (Andrews and Wood, 1986; Fargeas et al., 1988; Wood et al., 1987). These observations suggest that baclofen may alter gastrointestinal transit, particularly gastric emptying. However, this has not been previously measured. The aim of the current study was to determine the effects of baclofen on gastric emptying rates in mice using noninvasive [^{13}C] breath testing techniques.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice (aged >7 weeks) were housed in cages with continuous access to water and standard mouse pellets. All experiments followed the European Community guidelines and were approved by the Animal Ethics Committee of The Women's and Children's Hospital, North Adelaide, South Australia.

2.2. Effect of baclofen on gastric emptying

After an overnight fast, the mice were injected (intraperitoneal route) with 0.9% saline (control mice, $n=45$), 1 mg/kg baclofen [(±)-baclofen, Research Biochemicals International, Natick, MA, USA, $n=45$] or 2 mg/kg baclofen ($n=45$) at a volume of 10 ml/kg. Thirty minutes following the injection, 15 mice from each injection group were given one of three standard test meals, all used in previous experiments (Symonds et al., 2000, 2002). The meals were: (a) a nonnutrient liquid meal consisting of 100 μl of water containing 2% hydroxypropyl methyl cellulose (15,000 cp, Aldrich, Milwaukee, WI, USA) and 1 μl/ml [^{13}C]acetic acid (99% enrichment, Cambridge Isotope Laboratories, Andover, MA, USA), (b) a nutrient liquid meal

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consisting of 100 μl of 20% Intralipid (Kabi Pharmacia, Stockholm, Sweden) and 1 μl/ml [^{13}C]octanoic acid (99% enrichment, Cambridge Isotope Laboratories) and (c) a nutrient solid meal consisting of 0.1 g of baked egg yolk and 1 μl/g [^{13}C]octanoic acid. The effect on gastric emptying of a higher dose of baclofen (4 mg/kg) was assessed on the nonnutrient liquid ($n=10$). After the food consumption (the liquid meals were gavaged to the mice, whereas the solid consumption was voluntary), mice were placed in individual breath collection chambers and breath samples were collected for 120 min (liquid) and 150 min (solid) as previously described (Symonds et al., 2000).

2.3. Data analysis

The $^{13}CO_2$ content of breath samples was determined by an isotope ratio mass spectrometer (Europa Scientific, Crewe, England), and the measured $^{13}CO_2$ recovery in the breath was expressed as percentage excretion per hour of the given ^{13}C dose. Nonlinear regression analysis allowed calculation of gastric half emptying time ($t_{1/2}$) (Ghoos et al., 1993; Maes et al., 1994).

Data are expressed as means ± S.E.M. as all data displayed a normal distribution (Kolmogorov–Smirnov test). One-way analysis of variance with a Tukey post hoc test was used for the comparison of gastric emptying data between the different doses of baclofen and the saline injected group. $P<0.05$ was considered significant.

3. Results

Gastric half emptying time of liquid and solid test meals after injection with saline or 1–2 mg/kg baclofen are shown in Table 1. When compared to saline, baclofen delayed emptying of both nonnutrient and nutrient liquid meals at a dose of 2 mg/kg, but accelerated emptying of the solid meal, in a dose-dependent fashion, at doses of 1 and 2 mg/kg. Injection of 4 mg/kg baclofen produced a significant anaesthetic effect in 10 animals given this dose, therefore, only emptying of the nonnutrient liquid (the first test meal investigated) was assessed at this high dose. In the animals given this dose, the gastric emptying was delayed by 12-fold compared to mice injected saline ($t_{1/2}=200.01\pm55.16$ vs. 16.65 ± 1.22 min, respectively, $P<0.05$). The $^{13}CO_2$ excretion curves were also "double peaked" indicating a period of stasis during emptying of the meal.

Table 1
Gastric half emptying times for liquid and solid meals after injection of 0.9% saline, 1 mg/kg baclofen or 2 mg/kg baclofen

	Nonnutrient liquid	Nutrient liquid	Solid
Saline	16.65 ± 1.22	27.01 ± 2.53	145.87 ± 10.45
1 mg/kg baclofen	12.06 ± 0.64	26.70 ± 1.74	86.57 ± 9.23[a]
2 mg/kg baclofen	23.35 ± 3.12[b]	32.68 ± 1.40[b]	68.02 ± 5.53[a]

Data are means ± S.E.M. For each group, $n=15$.
[a] $P<0.001$ compared to saline-injected group.
[b] $P<0.05$ compared to saline-injected group.

4. Discussion

In this study, baclofen delayed gastric emptying of liquids, but paradoxically accelerated the emptying of solids. As solid and liquid meals are emptied by different mechanisms, these results suggest that baclofen may be producing differential effects on stomach motility during the different phases of gastric emptying.

Studies of the effect of baclofen on gastric motility have shown that baclofen both increases gastric tone (decreases gastric compliance) and potentiates rhythmical gastric contractions (Andrews et al., 1987; Andrews and Wood, 1986; Monroe and Hornby, 2002; Partosoedarso et al., 2001; Wood et al., 1987). The effect on gastric tone is likely to be due to decreased vagal drive to nonadrenergic noncholinergic motor neurones. However, the phasic contraction of the stomach is cholinergic in origin and, therefore, it has been suggested that baclofen may also increase vagal drive to cholinergic motor neurones by inhibiting nucleus of the solitary tract neurones which exert an inhibitory influence on the dorsal motor nucleus (Andrews et al., 1987). The paradoxical effects of baclofen on vagal drive and motility of different regions of the gastrointestinal tract may to some degree explain the differences in solid and liquid gastric emptying reported in the current study.

The acceleration of solid emptying in mice by baclofen was apparent at both doses evaluated. This effect was to be expected given the important role of antral peristalsis in the processes of trituration and emptying of ingested solids (Brown et al., 1993; Kelly, 1980). As previously mentioned, baclofen augments the amplitude of gastric contraction therefore we believe that this prokinetic effect is the main mechanism responsible for accelerated solid emptying.

Delayed liquid emptying by baclofen was an unexpected finding as gastric tone, the predominant force driving liquid gastric emptying (Brown et al., 1993; Kelly, 1980), should have increased. Delayed liquid emptying in a setting of increased gastric tone suggests that the resistance to transpyloric flow, which is predominantly due to tonic and phasic contractions of the pylorus, has also been significantly increased. The effect of baclofen on the motility of the pylorus has not been examined in previous animal studies. However, vagal stimulation has been shown to decrease pyloric tone and motility, while vagotomy and nitric oxide synthase inhibitors augment pyloric tone and motility (Ishiguchi et al., 2000; Lingenfelser et al., 1997; Malbert et al., 1995; Willis et al., 1996). These observations suggest that the pylorus is, like the lower oesophageal sphincter and stomach, under the influence of tonic vagal drive to nonadrenergic noncholinergic motor neurones. Therefore, a baclofen-induced decrease of vagal drive may augment pyloric tone and phasic contraction, which

would delay gastric emptying of liquids despite an increase in intragastric tone.

Differential effects on solid and liquid emptying have been previously reported with other conditions, for example, truncal or gastric vagotomy accelerates emptying of liquids but slows emptying of solids (Blat et al., 2001; MacGregor et al., 1977), and κ-opioid agonists (e.g., tifluadom) slow the emptying of liquids but speed the expulsion of solids (Gué et al., 1988). The fact that liquid and solid emptying are altered differently by baclofen, even though antropyloroduodenal motility is augmented, highlights the complexity of the mechanisms that regulate liquid and solid gastric emptying. It is well established that liquids and solids are emptied from the stomach at different times and by different mechanisms. During the early phase of gastric emptying of a mixed meal, antral motility is inhibited, pyloric tone increases and isolated pyloric pressure waves are stimulated (Houghton et al., 1988a; Houghton et al., 1988b). This pattern of motility serves to regulate the emptying of the liquid component of the meal. In contrast, trituration, mixing and emptying of the solid component of the meal occurs later and is facilitated by propagated antropyloroduodenal contractions (Houghton et al., 1988a). When solid and liquid emptying are considered in this way, it is very possible that augmentation of pyloric tone and isolated pyloric pressure waves in the early postprandial period would delay liquid emptying, while augmentation of propagated antropyoroduodenal contractions during the late phase of gastric emptying would accelerate solid emptying.

In conclusion, baclofen delays liquid emptying and accelerates solid emptying in mice, showing that the consistency of a meal is important in determining the net effect of the drug on gastric emptying. Any baclofen-induced alteration in gastric emptying, particularly a delay in emptying, may be important in the context of its use for the treatment of reflux. However, the doses used in the current study were higher that those shown to inhibit transient lower oesophageal relaxation and gastroesophageal reflux in humans (0.4–0.6 mg/kg) (Zhang et al., 2002), and at the lowest dose of 1 mg/kg, the only effect was an acceleration of solid emptying.

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