S.S. Hegde · A.G. Wong · M.R. Perry · P. Ku · T.M. Moy · M. Loeb · R.M. Eglen

5-HT\textsubscript{4} receptor mediated stimulation of gastric emptying in rats

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Abstract It is well documented that certain substituted benzamides, such as cisapride, and benzimidazolones, such as BIMU 8, enhance gastric emptying in rats. As these compounds possess 5-HT\textsubscript{3} antagonistic and 5-HT\textsubscript{4} agonistic properties, the precise mechanisms (5-HT\textsubscript{3} or 5-HT\textsubscript{4}) underlying their gastroprokinetic effects is still unclear. In the present study, we used SC 49518 (a benzamide and selective 5-HT\textsubscript{4} receptor agonist) and two selective 5-HT\textsubscript{4} receptor antagonists (RS 23597-190 and SB 204070) to elucidate the role of 5-HT\textsubscript{4} receptors in gastroprokinesis. SC 49518 (1–316 μg/kg; ip) produced significant and dose-dependent stimulation of gastric emptying in rats (ED\textsubscript{50} = 2.3 μg/kg; ip). SC 49518 also produced dose-dependent inhibition of bradycardia induced by 2-methyl 5-HT (von Bezold-Jarisch reflex) but with a 156 fold lower potency (ID\textsubscript{50} = 0.36 mg/kg; ip). The gastroprokinetic effects of SC 49518 (3–316 μg/kg; ip) were significantly antagonized by the selective 5-HT\textsubscript{4} receptor antagonist RS 23597-190 (0.1 mg/kg/min; iv). SB 204070 (0.003–1 mg/kg; ip), another selective 5-HT\textsubscript{4} receptor antagonist, produced dose-dependent inhibition of the gastroprokinetic effects of SC 49518 (10 μg/kg; ip), the inhibition attaining statistical significance at the dose of 0.1 mg/kg; ip. RS 23597-190 had no effects on gastric emptying per se whereas SB 204070 significantly increased gastric emptying by itself at 1 mg/kg; ip but not at 0.1 mg/kg; ip. These findings show, for the first time, that SC 49518, a selective 5-HT\textsubscript{4} receptor agonist, produces potent stimulation of gastric emptying in rats via a mechanism involving activation of 5-HT\textsubscript{4} receptors. It is suggested that a similar mechanism may account for the gastroprokinetic effects of other non-selective benzamides and benzimidazolones.

Key words 5-HT\textsubscript{4} receptors · Gastric emptying · Benzamides · SC 49518 · SC 53116 · RS 23597-190 · SB 204070

Introduction

Substituted benzamides, such as metoclopramide, cisapride, zacopride, renzapride and azabicycloalkyl benzimidazolones, such as BIMU 1 and BIMU 8, are endowed with 5-HT\textsubscript{3} receptor antagonistic and 5-HT\textsubscript{4} receptor agonistic properties (Dumuis et al. 1989; Turconi et al. 1991; see Ford and Clarke 1993 for review). These compounds have been shown to enhance gastric emptying in several species, including dogs and rats (Costall et al. 1985; Schiavone et al. 1990; Schiantarelli et al. 1990; Gullikson et al. 1991a; Rizzi et al. 1994). Several studies performed in dogs suggest that the gastroprokinetic effects in this species are mediated via agonism of 5-HT\textsubscript{4} receptors (Gullikson et al. 1993; Rizzi et al. 1994). Studies performed in rats are, however, more equivocal, with respect to the underlying mechanism. As the gastroprokinetic effects in rats can be mimicked by selective 5-HT\textsubscript{3} receptor antagonists, such as tropisetron (Schiavone et al. 1990), it has been hypothesized, by inference, that antagonism of 5-HT\textsubscript{3} receptors is the mechanism underlying the gastroprokinetic effects of benzamides and benzimidazolones in this species. Indeed, the gastroprokinetic potencies of these compounds in rats can be positively correlated to their potencies in inhibiting the von Bezold-Jarisch reflex (a 5-HT\textsubscript{3} receptor mediated effect) (Schiavone et al. 1990). However, support for this hypothesis has diminished somewhat in light of recent evidence showing that certain selective 5-HT\textsubscript{3} receptor antagonists, such as LY 277359, are devoid of gastroprokinetic effects.
properties in rats (Cohen et al. 1990). These findings question the involvement of 5-HT<sub>3</sub> receptors in gastroprokinesis. Consequently, alternate mechanisms, such as 5-HT<sub>4</sub> receptor agonism, have been offered to explain the gastroprokinetic effects of these compounds (Linnik et al. 1991). However, definitive demonstration of the involvement of 5-HT<sub>4</sub> receptors in gastroprokinesis has been precluded, thus far, by the lack of selective pharmacological probes for this receptor.

SC 53116, a pyrolizidone substituted benzamide, is a 5-HT<sub>4</sub> receptor agonist whose potency at 5-HT<sub>4</sub> receptors (as determined in the isolated rat oesophagus) exceeds its affinity for 5-HT<sub>3</sub> receptors by a factor of 6.4 fold (Flynn et al. 1992). This is in contrast to other benzamides, such as renzapride and zacopride, whose affinity for 5-HT<sub>3</sub> receptors exceeds their potency at 5-HT<sub>4</sub> receptors by a factor of greater than 60 fold (Flynn et al. 1992). Also, unlike metoclopramide and cisapride, SC 53116 possesses a low affinity for 5-HT<sub>3</sub>, D<sub>1</sub>, D<sub>2</sub> and α<sub>1</sub> receptors. This profile renders SC 53116 the most selective 5-HT<sub>4</sub> receptor agonist yet reported and also a valuable probe for evaluating the role of 5-HT<sub>4</sub> receptors in gastroprokinesis. A recent study showed that this compound, at a relatively high dose of 2.4 mmol/kg, ip, increased gastric emptying in rats, although no mechanistic evidence for the involvement of 5-HT<sub>4</sub> receptors was obtained (Valdovinos et al. 1993).

SC 49518 is a racemate compound composed of SC 53116 (the 1-S, 8-S enantiomer) and its less active 1-R, 8-R enantiomer (Gullikson et al. 1993). This compound has also been shown to behave as a selective 5-HT<sub>4</sub> receptor agonist (Gullikson et al. 1991b, 1993). At 5-HT<sub>4</sub> receptors in the rat oesophagus, SC 49518 is about 2.8 fold less potent than SC 53116. At 5-HT<sub>4</sub> receptors, SC 49518 has a lower affinity (IC<sub>50</sub> = 208 nM) than SC 53116 (IC<sub>50</sub> = 66 nM).

In the present study, we investigated the gastroprokinetic effects of SC 49518 and SC 53116 in rats. We chose to investigate the effects of SC 49518 more extensively than that of SC 53116 because of two reasons. Firstly, the in vitro and in vivo pharmacology of SC 49518 has been more extensively investigated in the literature. Secondly, the chemical separation of the two enantiomers is problematic. The limited experiments with SC 53116 reported in this study were performed with a limited sample of the drug donated generously by G.D. Searle, Illinois, USA.

We compared the gastroprokinetic potency of SC 49518 with its 5-HT<sub>4</sub> antagonistic potency (inhibition of the von Bezold-Jarisch reflex). Furthermore, using novel and selective 5-HT<sub>4</sub> receptor antagonists, such as RS 23597-197 (Eglen et al. 1993) and SB 204070 (Wardle et al. 1994), we assessed the contribution of 5-HT<sub>4</sub> receptors towards the gastroprokinetic effects of SC 49518. Portions of this work were presented at the annual meeting of 'American Society of Pharmacology and Experimental Therapeutics' (Ku et al. 1993).

Methods

Animals

Adult male Sprague-Dawley rats (Charles River, Wilmington, MA, USA), weighing 180-240 g, were used for this study. Animals were fasted overnight with water ad libitum. All experiments were conducted in accordance with guidelines established by the 'Institutional Animal Care and Use Committee' of Syntex Discovery Research.

Preparation of test meal for measurement of gastric emptying: 20 g of cellulose gum (Hercules Incor., Wilmington, Delaware) was slowly added to 200 ml of cold distilled water and mixed in a Waring blender at approximately 20,000 rpm. 2 beef bouillon cubes were dissolved in 100 ml water and then blended into the cellulose solution followed by 16 g of purified casein (Sigma Chemical Co., St Louis, MO), 8 g of powdered confectioners sugar, 8 g of corn starch and 1 g of powdered charcoal. The meal was refrigerated overnight to allow trapped air to escape. Prior to the assay, the meal was removed from the refrigerator to allow it to warm to room temperature.

Experimental protocols

Effects of SC 49518 on von Bezold-Jarisch reflex: Rats were anesthetized with ether. The left femoral vein of each rat was catheterized (with PE-50 tubing) to allow intravenous administration of drugs. The animals were allowed to recover and then placed in a restrainer. Heart rate was monitored via subdermal platinum electrocardiogram (ECG) electrodes connected to ECG/Biotach Gould amplifiers and recorded on a Gould 3800 recorder. Animals were challenged, intravenously, with 2-methyl 5-hydroxytryptamine (2-methyl 5-HT) (3.2-100 µg/kg) to determine the optimal submaximal dose (usually 10 µg/kg, ip) that would induce a reproducible bradycardic response (a decrease in heart rate of approximately 200 beats/min). Each animal was then challenged, at 30 min intervals, with the pre-determined dose of 2-methyl 5-HT. Fifteen minutes prior to each challenge of 2-methyl 5-HT, each animal was treated, intraperitoneally, with either vehicle (saline, 2 ml/kg) or ascending non-cumulative doses of SC 49518 (0.001-3.16 mg/kg). The inhibition of 2-methyl 5-HT-induced bradycardia was determined and expressed as a percentage of the control response to 2-methyl 5-HT.

Effects of SC 49518 on gastric emptying: Gastric emptying was measured using the method described by Droppleman et al. (1980). On the day of the experiment, animals were treated, intraperitoneally, with either vehicle (saline containing 4.5% 2-hydroxypropyl-β-cyclodextrin, 2 ml/kg) or the appropriate dose of SC 49518 (0.1-316 µg/kg). Thirty minutes later, each animal was dosed, orally, with 3 ml of a test semi-solid charcoal meal. Forty-five minutes later, the animals were sacrificed by CO<sub>2</sub> asphyxiation. The stomach was removed and weighed. Each stomach was cut open, rinsed, blotted dry and re-weighted. The amount of test meal remaining in the stomach was calculated from the difference between the full and empty stomach. This value was subtracted from the weight of 3 ml of test meal (average weight = 3.9 g) to yield the weight of meal emptied during the experimental period.

Effects of RS 23597-190 on the gastroprokinetic effects of SC 49518: As RS 23597-190 has an extremely short biological half-life (Eglen et al. 1993), this drug was infused intravenously into the animals. Animals were anesthetized with ether. The left femoral vein of each rat was catheterized for intravenous drug administration. The animals were allowed to recover from anesthesia and then...