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Full paper

RQ-00201894: A motilin receptor agonist causing long-lasting facilitation of human gastric cholinergically-mediated contractions

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ABSTRACT

The aim was to characterise RQ-00201894, a novel non-macrolide motilin agonist, using human recombinant receptors and then investigate its ability to facilitate cholinergic activity in human stomach. A reporter gene assay assessed motilin receptor function. Selectivity of action was determined using a panel of different receptors, ion channels, transporters and enzymes. Cholinergically-mediated muscle contractions were evoked by electrical field stimulation (EFS) of human gastric antrum. The results showed that RQ-00201894, motilin and erythromycin acted as full motilin receptor agonists (EC_{50}: 0.20, 0.11, 69 nM, respectively). In this function, RQ-00201894 had >90-fold selectivity of action over its ability to activate the human ghrelin receptor (EC_{50} 19 nM) and greater selectivity over all other receptors/mechanisms tested. In human stomach RQ-00201894 0.1–30 μM concentration-dependently increased EFS-evoked contractions (up to 1209%; pEC_{50} 6.0). At 0.1–10 μM this activity was usually prolonged. At higher concentrations (3–30 μM) RQ-00201894 also caused a short-lasting muscle contraction, temporally disconnected from the increase in EFS-evoked contractions. RQ-00201894 10 μM did not consistently affect submaximal contractions evoked by carbachol. In conclusion, RQ-00201894 potently and selectively activates the motilin receptor and causes long-lasting facilitation of cholinergic activity in human stomach, an activity thought to correlate with an ability to increase gastric emptying.

1. Introduction

The gastrointestinal (GI) hormone motilin is released from the upper gastrointestinal tract during hunger to evoke large, migrating contractions of the stomach which represent phase III of a pattern of GI movements during hunger, known as the migrating motor complex (MMC) (1). Further, this release of motilin may contribute to the mechanisms by which the sensation of hunger is initiated (2). Interestingly, however, motilin receptor agonists are also the target for developing new drugs which cause prolonged stimulation of gastric emptying of meals. This follows the discovery, in 1989, that the anti-biotic drug erythromycin is a non-selective motilin receptor agonist (3), followed by its subsequent 'off label' use as a gastric prokinetic agent (e.g. treating patients with gastroparesis, helping to control blood glucose levels in diabetic patients and increasing gastric emptying in patients receiving enteral feeding or requiring clearance of gastric contents before endoscopy or emergency surgery and its use in critically ill patients needing rapid intubation) (1). In addition, azithromycin, another antibiotic drug which activates the motilin receptor, is used to treat certain patients with gastro-oesophageal reflux, where increasing gastric emptying facilitates clearance of acid from the oesophagus (4). Nevertheless, there is concern that antibiotic drugs should not be used when there is no infection to treat, since this may contribute to growing anti-bacterial drug resistance (5). Erythromycin and azithromycin also have additional actions (e.g. inhibition of purinergic P2X channels and cytochrome 3A4 by erythromycin, and in rare cases, the potential for cardiac QT prolongation, arrhythmia and sudden cardiac death by erythromycin and azithromycin) (1, 4,

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6, 7). Finally, the optimal doses required to treat patients with gastroparesis are not rigorously established, with most investigators now using doses which are lower than those required to treat infections, an approach which appears to retain gastric prokinetic activity with minimal adverse events such as the induction of nausea (1).

Progress in identifying a non-antibiotic drug which acts selectively at the motilin receptor has been slow; as yet, no selective motilin receptor agonist has entered clinical practice. In part, the slow progress can be attributed to the complex macrolide structures of erythromycin, azithromycin and related molecules. This complexity greatly impedes the ability to undertake the precise structure-activity studies needed to optimise the selectivity of action, efficacy and duration of action of a motilin receptor agonist suitable for clinical use, in addition to the pharmacokinetic characteristics which enable such a molecule to become a successful drug (8). Thus, the identification of motilin receptor agonists with simpler structures, including those derived from dihydrotriazolopyridazine-1,3-dione-based amino acids (9) and more recently, the low molecular weight receptor agonist, camicinal (GSK962040 (10)) are now providing improved opportunities to develop effective motilin receptor agonists and successful drugs.

RQ-00201894 is a new non-macrolide, small molecule motilin receptor agonist (11). The pharmacology of RQ-00201894 is now defined in terms of its potency, efficacy and selectivity at the human motilin receptor. In addition, the ability of RQ-00201894 to facilitate gastric cholinergic motor transmission has been characterised using human isolated stomach. This is an important assay not just because it reflects the functions of the main excitatory motor nerves within the stomach, but because it also identifies motilin receptor agonists which induce either a long-lasting facilitation of cholinergic activity, more suitable for a gastric prokinetic drug or, like motilin itself, induce a shorter-lasting facilitation consistent with its role in mediating phase III MMC activity (12). Some of the data have previously been presented as meeting abstracts (13, 14).

2. Materials and methods

2.1. Activity of RQ-00201894, motilin and erythromycin at the human recombinant motilin receptor

CHO cells stably expressing both NFAT/β-lactamase reporter gene and human motilin receptor were seeded into 384-well black/clear-bottom plates. After overnight incubation, the culture medium was replaced by serum free medium at 37 °C for 1.5 h. The cells were then treated with the motilin receptor agonists at 37 °C for 4.5 h. Afterwards, CCF4/AM, a fluorogenic substrate (Invitrogen) was added and the cells incubated for 2 h. The fluorescence intensities were measured (Functional Drug Screening System; Hamamatsu Photonics) with excitation at 405 nm and emission at 465 and 540 nm. The concentration-response curves for RQ-00201894, motilin, and erythromycin were expressed as a percentage of the maximal control activity (50 μM erythromycin), and analyzed using GraphPad Prism (GraphPad Software) to obtain EC_{50} values. Data represent mean ± S.E.M. of three independent experiments performed in duplicate.

2.2. Evaluation of selectivity of action

The activity of a single concentration of RQ-00201894 (10 μM) was examined using a range of 64 different GPCRs, transporters, enzymes and ion channels expressed in native tissues and cell lines expressing human or rat recombinant proteins, provided by Cerep (Cerep, Paris, France), using standard radioligand binding, functional and enzyme assays. Specifically for the human ghrelin receptor, at which the effects of a range of concentrations of RQ-00201894 were studied, the receptors were transiently expressed in HEK293 cells and after seeding into 96 well plates (20,000 cells/100 μl/well) their function assessed using a Ca^{2+} influx assay with fluorescence intensity measured as above, as a ratio of emission intensities (510 nm from 340 nm excitation/510 from 380 nm). The concentration-response curves of RQ-00201894 and human ghrelin were expressed as percentage in proportion with maximal control activity (1 μM human ghrelin).

2.3. Human stomach

The method followed that which we have previously described (12). In brief, segments of stomach were obtained from patients undergoing surgery for obesity. The study was approved by the East London Research Ethics Committee 1, (REC reference number 10/H0703/71, SSA reference number 10/H0703/76), and written informed consent was obtained from all patients Tissues were transferred to the laboratory within 2 h after resection in Krebs' solution (mM: NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 5.6) equilibrated with 5% CO_2 and 95% O_2 and were used immediately or after overnight storage at 4 °C in fresh, oxygenated Krebs' solution.

The mucosa, muscularis mucosa and submucosal plexus were removed by blunt dissection. Strips of gastric antrum (3–5 × 15 mm) were cut approximately parallel to the circular muscle fibres and mounted in tissue baths (containing Krebs solution at 35 °C, gassed with 5% CO_2 in O_2) for measurement of changes in muscle tension using pre-calibrated isometric force transducers (AD Instruments, Chalgrove, UK) linked to a data acquisition system (Biopac Inc., CA, USA). After an initial application of 2 g tension the strips were allowed to recover for 60 min, during which the bath solutions were changed every 15 min. The strips were then stimulated via two parallel platinum ring electrodes connected to a stimulator (STG2008, Scientifica, Uckfield, UK). Electrical field stimulation (EFS) was applied at 5 Hz for 10 s using 50 V (c.200 mA) and 0.5 m bipolar pulse duration, repeated every 1 min. These parameters evoked monophasic, cholinergically-mediated contractions, attenuated by simultaneous activation of nitrergic inhibitory neurons (12), and were applied continuously until consistent responses were obtained (bath solution changed every 15 min).

In the experiments to examine the effects of RQ-00201894 on EFS-evoked contractions, each strip of stomach muscle was exposed to only a single concentration of RQ-00201894, which was then left in contact with the tissue for at least 60 min. The following measurements were taken:

1 Change in baseline muscle tension (expressed as a % of at least three pre-treatment EFS-induced contractions) and duration of any change,
2 Maximum change in EFS-evoked contractions (determined by measuring at least three EFS-induced responses at a given time-point, expressed as a percentage of the mean of at least three pre-treatment EFS-induced responses (100%)) and the time taken to achieve maximum response,
3 The length of time over which the maximum increase in EFS-evoked contraction was maintained during the continuous presence of the compound, measuring the time of fade of response if appropriate,
4 The occurrence of irregular contractions in response to EFS after application of RQ-00201894, recorded as a simple presence or absence.