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# Anti-emetic and emetic effects of erythromycin in *Suncus murinus*: Role of vagal nerve activation, gastric motility stimulation and motilin receptors

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ABSTRACT

Paradoxically, erythromycin is associated with nausea when used as an antibiotic but at lower doses erythromycin activates motilin receptors and is used to treat delayed gastric emptying and nausea. The aim of this study was to characterise pro- and anti-emetic activity of erythromycin and investigate mechanisms of action. Japanese House musk shrews (*Suncus murinus*) were used. Erythromycin was administered alone or prior to induction of emesis with abnormal motion or subcutaneous nicotine (10 mg/kg). The effects of erythromycin and motilin on vagal nerve activity and on cholinergically mediated contractions of the stomach (evoked by electrical field stimulation) were studied *in vitro*. The results showed that erythromycin (1 and 5 mg/kg) reduced vomiting caused by abnormal motion (e.g., from $10.3 \pm 1.8$ to $4.0 \pm 1.1$ emetic episodes at 5 mg/kg) or by nicotine (from $9.5 \pm 2.0$ to $3.1 \pm 2.0$ at 5 mg/kg), increasing latency of onset to emesis; lower or higher doses had no effects. When administered alone, erythromycin 100 mg/kg induced vomiting in two of four animals, whereas lower doses did not. *In vitro*, motilin (1, 100 nM) increased gastric vagal afferent activity without affecting jejunal afferent mesenteric nerve activity. Cholinergically mediated contractions of the stomach (prevented by tetrodotoxin 1 μM or atropine 1 μM, facilitated by L-NAME 300 μM) were facilitated by motilin (1–100 nM) and erythromycin (10–30 μM). In conclusion, low doses of erythromycin have anti-emetic activity. Potential mechanisms of action include increased gastric motility (overcoming gastric stasis) and/ or modulation of vagal nerve pathways involved in emesis, demonstrated by first-time direct recording of vagal activation by motilin.



## 1. Introduction

Paradoxically, when used as an antibiotic erythromycin is associated with side-effects of nausea and vomiting (Catnach and Fiarclough, 1992; Boivin et al., 2003), yet because of its ability to stimulate gastric emptying, erythromycin is used to treat conditions where nausea is a symptom (e.g., diabetic gastroparesis: Dibaise and Quigley, 1999; Gonlachanvit et al., 2003; Maganti et al., 2003; Ritz et al., 2005). These actions are usually observed at different doses. Thus, the doses used to treat patients with delayed gastric emptying (often associated with nausea; Cherian and Parkman, 2011) are lower than those given for antibiotic use (Desautels et al., 1995; Sanger, 2008), minimising nausea, stomach cramping, early satiety (Cuomo et al., 2006), tolerance to repeat dosing (Dibaise and Quigley, 1999; Gonlachanvit et al., 2003; Maganti et al., 2003; Dhir and Richter, 2004; Hunter et al., 2005; Ritz et al., 2005) and loss of therapeutic benefit (Richards et al., 1993).

Erythromycin is also a motilin receptor agonist (Feighner et al., 1999) and the above paradoxical actions are potentially reproduced in experiments with human (Broad et al., 2012) and rabbit (Dass et al., 2003; Depoortere et al., 2003; Jarvie et al., 2007; Sanger et al., 2009) isolated stomach. Here, low concentrations of motilin receptor agonists facilitate cholinergic activity to increase gastric motility, whereas higher concentrations hyper-stimulate cholinergic function and directly contract the muscle, promoting nausea by contracting the gastric fundus (Bruley Des Varannes et al., 1995) and inducing prolonged (Tack et al., 1992), non-propulsive (Coulie et al., 1998) hypermotility of the antrum.

It is unlikely that erythromycin acts within the brain, where functional motilin receptors have not been identified (apart from some studies in rodents, treated with caution because of the absence of a functional motilin system in rodents; Sanger et al., 2011). However, erythromycin could influence emesis by modulating vagal nerve activity (Andrews and Sanger, 2002). This is

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suggested by reports that vagotomy inhibits motilin- or erythromycin-induced stimulation of canine gastric motility and release of certain hormones (Inatomi et al., 1996; Mochiki et al., 1997; Mathis and Malbert, 1998; Suzuki et al., 1998).

Studies on either motilin or emesis are not possible in rodents (mice, rats, guinea-pigs; e.g., Bassil et al., 2005) in which only motilin and motilin receptor pseudogenes are identified, a degeneration correlating with unusual digestive physiology, including loss of ability to vomit (Sanger et al., 2011). We therefore studied emesis in Japanese House musk shrews (*Suncus murinus*), phylogenetically closer to humans than other small laboratory animals (Hoyle et al., 2003) and extensively used in emesis research (Ueno et al., 1988; Okada et al., 1995; Javid and Naylor, 2001, 2006); in these animals, motilin receptor mRNA and motilin have been identified, respectively, in the nodose ganglia (Suzuki et al., 2012) and gastrointestinal endocrine cells (Kanamori et al., 1989, 1990). The aims were to model the effects of erythromycin on emesis and investigate mechanisms of action by studying vagal nerve and gastric neuromuscular activities *in vitro*, representing respectively, the gut-brain and the enteric nerve pathways by which gastric stasis and nausea can be influenced.

## 2. Materials and methods

### 2.1. Animals and housing conditions

Adult Japanese House Musk shrews (*S. murinus*) of either sex from the Bradford University strain were used; animals used for the vomiting experiments were not re-used for the *in vitro* experiments. The shrews were individually housed and allowed food (AQUATIC 3, trout pellets) and water *ad libitum*. Animals were also fed with mealworms or cat food three times per week. The floor of the cages were covered with sawdust and cleaned twice a week. The animal room was maintained at humidity between 45 and 55% at 19–21 °C and on a normal light-dark cycle.

### 2.2. Studies on vomiting

All experimental procedures were in compliance with the UK Animals (Scientific Procedures) Act 1986, and were carried out using age-matched animals of either sex (female $36.5 \pm 1.2$ g; adult male $71.1 \pm 1.2$ g). Prior to studying the effects of erythromycin on vomiting, the effects of this drug were examined when given alone. In these experiments, animals received intraperitoneal (i.p.) injections of erythromycin at 0.1, 1.0, 5.0, 10.0 or 100.0 mg/kg, or vehicle as a single challenge. Immediately after, each animal was placed individually in a transparent cage ($100W \times 150L \times 150H$ mm) of six linked units and observed for any behavioural change.

To study the effects of erythromycin on vomiting induced by abnormal motion, a horizontal motion stimulus of 1.0 Hz and 40.0 mm amplitude of shaking was commenced for 10 min. Previous experiments showed that these parameters were suitable to induce a reliable and reproducible emetic response (Javid and Naylor, 1999). In all experiments, the number of emetic episodes (defined as productive vomiting or as dry retching) and the latency of onset to the first emetic episode were recorded. It should be noted that the animals were kept and tested in exactly the same environment to obviate confounding differences of olfactory, visual and other cues. All the experiments were conducted at the same time every day. Animals received erythromycin at 0.1, 1.0, 5.0, 10.0 or 100.0 mg/kg (i.p.) or vehicle 5 or 45 min prior to the motion stimulus and were observed over a 90 min period for any overt behavioural changes.

To study the effects of erythromycin on vomiting induced by nicotine, it was first necessary to select the appropriate dose of nicotine. In these experiments, animals received subcutaneous (s.c.) injections of nicotine at 5.0 or 10.0 mg/kg, or vehicle alone as a single challenge and were observed for any behavioural changes as described above. Following selection of the dose of nicotine which provided a reproducible response, and in separate experiments, animals were injected with either vehicle or erythromycin (0.1, 1.0, 5.0 or 10.0 mg/kg, i.p.) 45 min prior to administration of nicotine (10.0 mg/kg, s.c.) and were then observed over a 30 min observation period following administration of nicotine.

### 2.3. Studies in vitro

Animals were euthanized by rising concentrations of $CO_2$, followed by cervical dislocation or exsanguination in accordance with Schedule 1 of the UK Animals (Scientific Procedures) Act 1986. All efforts were made to minimise the number of animals used.

### 2.4. Studies using the isolated vagus nerve

Jejunum and stomach with oesophagus attached, were removed and placed in ice cold Krebs solution (NaCl 121.5, $CaCl_2$ 2.5, $NaH_2PO_4$ 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.6 mM) supplemented with nifedipine (10 μM), atropine (10 μM) and indomethacin (3 μM), bubbled continuously with 5% $CO_2$ in $O_2$. The stomach was pinned flat in a sylgard lined tissue bath and transected off-centre to the midline such that the mucosa of the ventral surface was face up and the integrity of the oesophagus, and the vessels and nerves traversing the lesser curvature of the stomach were preserved. The preparation was perfused continuously with Krebs solution supplemented as above (flow rate 7 ml/min, temperature 32–34 °C) and a nerve fascicle of the ventral gastric branch of the vagus dissected free caudal to the accessory coeliac branch of the vagus. Correct isolation of the ventral gastric branch of the vagus was confirmed by probing the mucosal surface with cotton bud tips to identify receptive fields. In separate experiments, a length of jejunum (~3 cm long) with mesenteric arcades attached was transected off-centre to the mesenteric border and pinned mucosal surface up in a sylgard-lined tissue bath. The tissue was perfused continuously with Krebs solution supplemented as above and a mesenteric nerve bundle carefully dissected free.

Recordings of nerve activity were facilitated using suction electrodes made from pulled borosilicate glass capillary tubes and back-filled with bath Krebs. Recordings of nerve activity were amplified (5 K) and filtered (band pass 100–1300 Hz, digital 50 Hz), and the resulting signal passed through a spike processor and oscilloscope to facilitate action potential counting using a level indicator set twice the back ground noise. The filtered nerve activity and action potential "events" were digitised using a 1401 analogue to digital converter and displayed/analysed on a computer using Spike2 software. Following a 30–60 min stabilisation period the response to bath perfusion with motilin (10 ml) was examined. In some preparations the effect of vehicle perfusion (supplemented Krebs buffer) was examined up to 30 min prior to administration of motilin. Additionally in some preparations the response to a second application of motilin was examined a minimum of 30 min after the first motilin application. Mean nerve activity was determined for consecutive 60 s periods of time for both stomach and jejunal preparations using a home-made semi-automated script. Potential increases or decreases in mean nerve activity were examined by subtracting the peak 60 s mean firing rate up to 5 min prior to application of vehicle or