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Inhibition of native 5-HT$_3$ receptor-evoked contractions in guinea pig and mouse ileum by antimalarial drugs

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ABSTRACT

Quinine, chloroquine and mefloquine are commonly used to treat malaria, however, with associated gastrointestinal (GI) side-effects. These drugs act as antagonists at recombinant 5-HT$_3$ receptors and modulate gut peristalsis. These gastrointestinal side effects may be the result of antagonism at intestinal 5-HT$_3$ receptors. Ileum from male C57BL/6 mice and guinea pigs was mounted longitudinally in organ baths. The concentration–response curves for 5-HT and the selective 5-HT$_3$ agonist 2-Me-5-HT were obtained with 5-HT ($pEC_{50}=7.57\pm0.33$, 12) more potent ($P=0.004$) than 2-Me-5-HT ($pEC_{50}=5.45\pm0.58$, $n=5$) in mouse ileum. There was no difference in potency of 5-HT ($pEC_{50}=5.42\pm0.15$, $n=8$) and 2-Me-5-HT ($pIC_{50}=5.01\pm0.55$, $n=11$) in guinea pig ileum ($P>0.05$). Quinine, chloroquine or mefloquine was applied for 10 min and inhibitions prior to submaximal agonist application. In mouse ileum, quinine, chloroquine and mefloquine antagonised 5-HT-induced contractions ($pIC_{50}=4.9\pm0.17$, $n=7$; 4.76 ± 0.14, $n=5$; 6.21 ± 0.2, $n=4$, correspondingly) with mefloquine most potent ($P<0.05$). Quinine, chloroquine and mefloquine antagonised 2-me-5-HT-induced contractions ($pIC_{50}=6.35\pm0.11$, $n=8$; 4.64 ± 0.2, $n=7$; 5.11 ± 0.22, $n=6$, correspondingly) with quinine most potent ($P<0.05$). In guinea-pig ileum, quinine, chloroquine and mefloquine antagonised 5-HT-induced contractions ($pIC_{50}=5.02\pm0.15$, $n=6$; 4.54 ± 0.1, $n=7$; 5.32 ± 0.13, $n=5$) and 2-me-5-HT-induced contractions ($pIC_{50}=4.62\pm0.25$, $n=5$; 4.56 ± 0.14, $n=6$; 5.67 ± 0.12, $n=4$) with chloroquine least potent against 5-HT and mefloquine most potent against 2-me-5-HT ($P<0.05$). These results support previous studies identifying anti-malarial drugs as antagonists at recombinant 5-HT$_3$ receptors and may also demonstrate the ability of these drugs to influence native 5-HT$_3$ receptor-evoked contractile responses which may account for their associated GI side-effects.

1. Introduction

Enterochromaffin (EC) cells, within the epithelial layer of the gastrointestinal tract, release 5-HT and account for 90% of the body's store of 5-HT (Bueno, 2005). A variety of 5-HT receptors located on intestinal cells modulate peristalsis (Tuladhar et al., 2003, 1997, 2000) and secretions (Turvill et al., 2000). These include 5-HT$_{1A}$, 5-HT$_3$ 5-HT$_4$, and 5-HT$_7$ receptors (Hoyer et al., 2002). 5-HT$_3$ receptors play an important role in the excitability of the enteric nervous system, contributing to fast excitatory post-synaptic potentials in neurones of the myenteric and submucosal plexuses (Galligan, 2002, 2000; Michel et al., 2005). 5-HT$_3$ receptors are distributed throughout the human, guinea pig, rat and mouse intestine (Butler et al., 1990; Champaneria et al., 1992; Chetty et al., 2006, 2008; Gaddum and Picarelli, 1957; Kapeller et al., 2011), and play a pivotal role in modulating intestinal motility (Chetty et al., 2006; Liu et al., 2011; Mayer et al., 2006). Additionally, 5-HT$_3$ receptor antagonists such as ramosetron have been indicated for diarrhoea-predominant irritable bowel syndrome by blocking intestinal 5-HT$_3$ receptors (Lee et al., 2011).

The principal side effects of quinine, chloroquine and mefloquine include gastrointestinal disturbances such as nausea, vomiting, diarrhoea and constipation (Barrett et al., 1996; Fogh et al., 1988; White, 1992). This may be partially due to an interaction with receptors or ion channels expressed within the gut and the enteric nervous system. Quinine is known to block voltage-gated K^+ channels (Schmalz et al., 1998) and chloroquine may indirectly modulate large Ca^{2+}-activated K^+ channels (BKca) in the ileum (Jing et al., 2013). Quinine, chloroquine, and mefloquine have also been shown to act as antagonists at human and mouse recombinant 5-HT$_{3A}$ homo-oligomeric receptors expressed in Xenopus oocytes (Thompson et al., 2007; Thompson and Lummis, 2008).

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Additionally, quinine, chloroquine and mefloquine can displace [^{3}H]granisteron binding to mouse recombinant 5-HT$_3$ receptors (Thompson et al., 2007) indicating that these anti-malarial drugs directly bind to the 5-HT binding site on the receptor. We hypothesise that these anti-malarial drugs, quinine, chloroquine and mefloquine will also act as antagonists at native 5-HT$_3$ receptors in the small intestine, and by doing so may significantly attenuate 5-HT$_3$-mediated contractions. In an attempt to investigate this hypothesis, we have utilised both mouse and guinea-pig isolated ileum preparations and examined the ability of the anti-malarial compounds, quinine, chloroquine and mefloquine to antagonise 5-HT and 5-HT$_3$ mediated contractions. The rationale for using both mouse and guinea pig ileum being that the action of anti-malarial compounds at recombinant mouse 5-HT$_{3A}$ homo-oligomeric receptors has been evaluated previously (Thompson et al., 2007) and the guinea-pig 5-HT$_{3A}$ subunit (Lankiewicz et al., 2000) has a 85% sequence homology with its human counterpart with relatively similar agonist pharmacology to human recombinant 5-HT$_{3A}$ receptors (Belelli et al., 1995).

2. Materials and methods

2.1. Preparation of tissues

Male C57BL/6 mice (25–35 g; Charles River Laboratories, Margate, UK) were killed by cervical dislocation and the ileum was excised 2 cm before the ileo-caecal junction and placed in Tyrode's solution (in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.42, $NaHCO_3$ 12.0, Glucose 5.5, pH 7.4). Segments of whole ileum (3–4 cm) were then carefully mounted longitudinally in 50 ml water jacketed organ baths containing Tyrode's solution continuously aerated with 95% O_2/5% CO_2 and kept at 35–37 °C. The ileum segments were allowed to equilibrate for 30 min whilst mechanically attached to a force transducer with a resting tension of 0.5 g. The contractile responses were recorded by the forced transducer and visualised by means of a chart recorder.

Portions of guinea pig ileum were obtained from adult male guinea pigs (200–300 g, Charles River, Laboratories, Margate, UK). The ileum was cut into 3.5–4 cm and mounted in a similar manner to that of mouse ileum. The tissues were equilibrated for 30 min followed by an initial application of acetylcholine (ACh; 1 μM) to establish the integrity of the tissue at the start of the experiment. All procedures involving animals were approved by the University of Kent Animal Welfare and Ethical Review Body in accordance with the UK Animals (Scientific Procedures) Act (1986).

2.2. Drugs

Acetylcholine, chloroquine, quinine (Sigma Aldrich, Poole, UK), 5-hydroxytryptamine hydrochloride (Tocris Bioscience, Bristol, UK), 2-methyl-5-HT (Tocris Bioscience, Bristol, UK) were dissolved in Tyrode's solution. Mefloquine (Sigma Aldrich, Poole, UK) was dissolved in 50% dimethyl sulfoxide then diluted in Tyrode's solution to a final dimethyl sulfoxide concentration of $\leq$0.3%. 5-HT and 2-methyl-5-HT were applied to the serosal layer of the ileum and responses recorded for 30 se which was sufficient for capturing the maximal contraction evoked from the drug. The Tyrode's solution was then flushed and the organ bath filled with fresh Tyrode's solution. The ileum was then maintained for 10 min prior to application of the next agonist concentration. For the anti-malarial (antagonist) compounds, application was also made to the serosal layer, however the ileum remained in contact with the anti-malarial compounds for 10 min. Following this 10 min interval, agonist was applied in the manner described above and the agonist evoked response recorded. The application of antagonists was not initiated until at least two agonist baseline responses (mm) in the absence of an antagonist did not differ greater than 5%.

2.3. Analysis of results

To construct the 5-HT and 2-me-5-HT agonist concentration–response curves, individual agonist-evoked contractile response heights (mm) were normalised to the maximal contraction height for each ileum. The mean normalised responses ± S.E.M. for each agonist concentration in a series were iteratively fitted using GraphPad Prism (version 6, Iowa, USA) to the non-linear regression equation

$$y = E_{min} + \frac{(E_{max} - E_{min})}{1 + 10^{(\log EC_{50} - L)n_H}}$$

where E_{min} is the baseline contraction, E_{max} is the maximal agonist-evoked contraction, EC_{50} is the concentration of the agonist required to produce 50% of the maximal contraction, L is the log of the agonist concentration and n_H is the Hill slope. Agonist potency was expressed as EC_{50} and pEC_{50} is the negative log of the EC_{50}.

For antagonist experiments, baseline agonist response heights (mm) were measured and antagonist effects were measured as a % of the mean baseline agonist response for each ileum preparation. The relationships between increasing antimalarial (antagonist) concentration and % inhibition of agonist concentration-evoked contractions were iteratively fitted using GraphPad Prism (version 6, Iowa, USA) to the non-linear regression equation

$$y = \frac{100}{1 + 10^{(\log IC_{50} - L)n_H}}$$

where IC_{50} is the concentration of the antagonist required to reduce to 50% of the agonist contraction, L is the log of the antagonist concentration and n_H is the Hill slope. Agonist potency was expressed as IC_{50} and pIC_{50} is the negative log of the IC_{50}.

5-HT and 2-me-5-HT pEC_{50}s were compared independently for mouse and guinea pig by a Student's t-test, whilst pIC_{50}s for each antagonist against 5-HT and 2-me-5-HT were compared by a oneway analysis of variance (ANOVA) followed by post-hoc analysis (Tukey's t). Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Mouse tissue

Both 5-HT and 2-methyl-5-HT were able to evoke concentration-dependent contractions in mouse ileum tissue 5-HT which was significantly more potent in its ability to induce contraction of the ileum than 2-methyl-5-HT (Fig. 1). Potency (expressed as pEC_{50}) for 5-HT was 7.57 ± 0.33 (n=12), whilst potency for 2-methyl-5-HT was 5.45 ± 0.58 (n=5), which was significantly greater than potency for 5-HT when compared to the selective 5-HT$_3$ agonist (Student's t-test, t=3.36, df=15, P=0.004). With increasing concentrations, quinine was able to successfully antagonise 5-HT-induced (25 nM) contractions in mouse ileum (pIC_{50}=4.9 ± 0.17, n=7, Fig. 2A) with complete block at 300 μM. Chloroquine also antagonised 5-HT induced contractions (pIC_{50}=4.76 ± 0.14, n=5, Fig. 2B) as did mefloquine (pIC_{50}=6.21 ± 0.2, n=4, Fig. 2C). A one-way analysis of variance revealed a statistically significant difference in the potency of the antimalarials to act as antagonists of the 5-HT mediated contractions [F(2, 13)=17.90, $P < 0.001$] with mefloquine acting as the most potent antagonist of 5-HT mediated contractions (Tukey's t, $P < 0.05$). A 10 min wash was sufficient to reinstate 5-HT-evoked

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