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Anatomy and function of group III metabotropic glutamate receptors in gastric vagal pathways

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Abstract

Metabotropic glutamate receptors (mGluR) are classified into groups I (excitatory), II and III (inhibitory) mGluR. Activation of peripheral group III mGluR (mGluR4, mGluR6, mGluR7, mGluR8), particularly mGluR8, inhibits vagal afferent mechanosensitivity in vitro which translates into reduced triggering of transient lower oesophageal sphincter relaxations and gastroesophageal reflux in vivo. However, the expression and function of group III mGluR in central gastrointestinal vagal reflex pathways is not known. Here we assessed the expression of group III mGluR in identified gastric vagal afferents in the nodose ganglion (NG) and in the dorsal medulla. We also determined the central action of the mGluR8a agonist *S*-3,4-DCPG (DCPG) on nucleus tractus solitarius (NTS) neurons with gastric mechanosensory input in vivo. Labelling for mGluR4 and mGluR8 was abundant in gastric vagal afferents in the NG, at their termination site in the NTS (subnucleus gelatinosus) and in gastric vagal motorneurons, while labelling for mGluR6 and mGluR7 was weaker in these regions. DCPG (0.1 nmol or 0.001–10 nmol i.c.v.) inhibited or markedly attenuated responses of 8/10 NTS neurons excited by isobaric gastric distension with no effect on blood pressure or respiration; 2 NTS neurons were unaffected. The effects of DCPG were significantly reversed by the group III mGluR antagonist MAP4 (10 nmol, i.c.v.). In contrast, 4/4 NTS neurons inhibited by gastric distension were unaffected by DCPG. We conclude that group III mGluR are expressed in peripheral and central vagal pathways, and that mGluR8 within the NTS selectively reduce excitatory transmission along gastric vagal pathways.

Keywords: Electrophysiology; Group III metabotropic glutamate receptors; Immunohistochemistry; Gastric vagal afferent; Nucleus tractus solitarius

1. Introduction

A significant population of gastric vagal afferents respond to distension and contraction of smooth muscle (Page and Blackshaw, 1998). They participate in a wide range of reflexes and behavioural responses, including transient lower oesophageal sphincter relaxation (TLESR), regulation of gastric emptying, satiety, nausea and vomiting. TLESR is the predominant mechanism of gastroesophageal acid reflux (Dent, 1998; Holloway and Dent, 1990), and thus the target mechanism in the treatment of gastroesophageal reflux disease (Blackshaw, 2001; Holloway, 2001). There is good evidence that TLESR are triggered by gastric distension and are mediated by a vagal pathway involving gastric tension receptors, nuclei in the central nervous system (CNS) and vagal inhibitory pathways to the LES smooth muscle (Mittal et al., 1995). This pathway expresses a wide array of neurotransmitter receptors, suggesting it may be rich in molecular targets that reduce triggering of TLESR. Agonists of $GABA_B$, cannabinoid and μ-opioid receptors act at various points along vagal and CNS pathways to potently inhibit TLESR (Blackshaw et al., 1999; Lehmann et al., 1999, 2000; Lidums et al., 2000; Penagini and Bianchi, 1997). Importantly, these actions may be associated with reduced reflux and reflux symptoms in gastroesophageal reflux disease patients (Ciccaglione and Marzio, 2003).

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Glutamate is the predominant neurotransmitter in the CNS and in addition to the well-recognized fast synaptic transmission at ionotropic glutamate receptors (iGluR), glutamate also mediates slow synaptic neurotransmission within the CNS at metabotropic glutamate receptors (mGluR) (Foley et al., 1998; Glaum and Miller, 1992). These diverse effects are mediated by group I mGluR (mGluR1 and 5) which cause slow depolarization via positive coupling to phospholipase-C, and group II (mGluR2 and 3) and III mGluR (mGluR4, 6, 7 and 8) which cause slow hyperpolarization via negative coupling to adenylate cyclase and via altered calcium and potassium currents (see review by Cartmell and Schoepp, 2000). In this respect group III mGluR share many features with inhibitory $GABA_B$ receptors, but offer more amenable pharmacology and potential for therapeutic intervention.

There is anatomical evidence that the majority of vagal afferents innervating gastric muscle express group III mGluR and actively transport mGluR4, 7 and 8 to their peripheral endings (Hoang and Hay, 2001; Li et al., 1996; Page et al., 2005b). These afferents also project centrally to the NTS which has been shown to express group III mGluR in rat (Hay et al., 1999; Kinoshita et al., 1998; Pamidimukkala et al., 2002). However, rats do not exhibit TLESR and data from species known to display TLESR, such as ferrets, is lacking. Importantly, our group has shown that group III mGluR agonists in general, and the selective mGluR8 agonist (S)-3,4-DCPG (DCPG) in particular, powerfully reduce the mechanosensitivity of peripheral gastroesophageal vagal afferent endings in ferrets and mice (Page et al., 2005b) and inhibit TLESR in a conscious ferret model (Frisby et al., 2005).

Transmission of signals in the NTS is principally glutamatergic, and group III mGluR have been shown to depress vagal transmission at first-order synapses via actions at predominantly pre-synaptic receptors (Chen et al., 2002; Glaum and Miller, 1993; Liu et al., 1998; Paton et al., 2000). We hypothesized therefore that in addition to reducing TLESR by modulating responses of vagal afferent endings to gastric distension peripherally, group III mGluR also reduce transmission of gastric vagal signals at a central site.

We tested this hypothesis by mapping the anatomy and group III mGluR expression of gastric vagal pathways using retrograde tracing and immunohistochemistry in ferrets. We also assessed in vivo responses of NTS neurons with input from gastric distension in the absence or presence of the mGluR8a agonist DCPG; the reversibility of these NTS responses was also tested with the group III mGluR antagonist MAP4. These lines of study are crucial for understanding the role of group III mGluR in gastric vagal pathways, and their potential for therapeutic targeting in patients with gastroesophageal reflux disease.

2. Methods

2.1. Animal preparation

Experiments were performed on adult (1.0–1.5 kg) male ferrets. All studies were performed in accordance with the Australian code of practice for the care and use of animals for scientific purposes and with the approval of the Animal Ethics Committees of the Institute of Medical and Veterinary Science (Adelaide, Australia) and the University of Adelaide. Animals had free access to water and a standard carnivore diet and were fasted overnight prior to experimentation.

2.2. Retrograde tracing

This protocol has been described previously (Page et al., 2005a; Smid et al., 2001; Young et al., 2007). Ferrets were anaesthetized with isoflurane (2–3% in oxygen) and 20 μl of cholera toxin B subunit conjugated to Alexa Fluor 555 (0.5% in 0.1 M phosphate buffer pH 7.4, CTB-AF555; Invitrogen, Mount Waverley, Australia) or to FITC (CTB-FITC; Sigma Aldrich, Castle Hill, Australia) was injected into the proximal stomach subserosa using a 26-gauge Hamilton syringe ($n = 5$ per tracer). Multiple equally spaced injections of 2–4 μl were made around the full circumference of the proximal stomach, approximately 1 cm from the oesophagogastric junction (total volume 20 μl). The injection sites were dried, the laparotomy incision closed and antibiotic (Terramycin 10 mg/kg) and analgesic (Metacam 2.5 mg/kg) administered subcutaneously. All ferrets recovered well from surgery and after 4 days were deeply anesthetized (pentobarbitone, 60 mg kg^{-1} i.p.), perfuse-fixed with 4% paraformaldehyde and the NG and brainstem removed, fixed overnight at 4 °C then cryoprotected in 30% sucrose at 4 °C for 24–48 h. Frozen serial transverse sections (20 μm) were then cut through the rostrocaudal axis of the NG and brainstem for immunohistochemistry.

Control experiments for detecting tracer spread were also performed, by separate intraperitoneal injection of either tracer (20 μl), and by assessment of tracer distribution in anterior and posterior flat sheets of the stomach at 2 h, and 1 and 2 days following tracing in two ferrets for each time course. Control experiments to assess central labelling of vagal afferent terminals were also performed in two ferrets that underwent a selective anterior subdiaphragmatic vagotomy prior to injection of CTB-AF555 into gastric muscle.

2.3. Immunohistochemistry

Using immunohistochemical protocols previously described in detail (Young et al., 2007), immunoreactivity for group III mGluR was detected in traced NG and brainstem sections using rabbit anti-rat polyclonal primary antibodies from a number of suppliers (1–2 for each target, Table 1). Primary antibody was visualized by a goat anti-rabbit secondary antibody conjugated to Alexa Fluor 488 or 546 (Invitrogen, Mount Waverley, Australia) and selected to contrast the tracer used. Sections were air dried at room temperature (RT) for 10 min and rinsed twice in PBS + 0.2% Triton X-100 (Sigma Aldrich, PBS-T, pH 7.4) to facilitate antibody penetration. Primary antibodies were diluted in 10% PBS-T according to vendor's recommendations and results of positive labelling assays (Table 1 and described below) and incubated overnight at 4 °C; unbound antibody was then removed with PBS-T washes and slides incubated for 1 hr at RT with secondary antibody (1:200 in PBS-T). Slides were given final PBS-T washes, drained and mounted with ProLong

Table 1
Antibodies used for localization and detection of group III mGluR

Receptor	Sequence (dilution)	Supplier	Category No.
mGluR4a	Residues 893–912	Upstate Biotechnology	06-765
	Within terminal 200 residues (1:2000)	Zymed Laboratories	51-3100
mGluR6	Residues 859–871	Neuromics	RA13105
mGluR7	Residues 899–912	Upstate Biotechnology	07-239
mGluR8	Residues 889–908	Upstate Biotechnology	07-174
	Residues 894–908 (guinea-pig 1:2000)	Chemicon–Millipore	AB5362

All primary antibodies are used at a dilution of 1:200 unless indicated, and in accord with vendor's recommendations and outcomes of preliminary control assays. Antibodies were directed to C-termini, and were rabbit anti-rat antibodies unless indicated.