Metabotropic Glutamate Receptors Inhibit Mechanosensitivity in Vagal Sensory Neurons

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Background & Aims: **Inhibitory G-protein-coupled receptors have demonstrated potential in treatment of gastroesophageal reflux disease (GERD) through actions on vagal afferent signaling. Metabotropic glutamate receptors (mGluR) belong to this receptor family and have great pharmacologic and molecular diversity, with 8 subtypes. We investigated mGluR in the vagal system of humans and other species.** ***Methods:*** **Expression of mGluR1-8 in human, dog, ferret, and rodent nodose ganglia was investigated by reverse-transcription polymerase chain reaction. mGluR1-8 immunohistochemistry was performed in combination with retrograde tracing of vagal afferents from ferret proximal stomach to nodose ganglia. Transport of mGluR peripherally was investigated by vagal ligation, followed by immunohistochemistry. Glutamate receptor pharmacology of ferret and rodent gastroesophageal vagal afferents was investigated by testing single fiber responses to graded mechanical stimuli during drug application to their peripheral endings.** ***Results:*** **Messenger RNA for several mGluR was detected in the nodose ganglia of all species. Retrograde tracing indicated that ferret gastric vagal afferents express mGluR protein. Accumulation of immunoreactivity proximal to a ligature showed that mGluR were transported peripherally in the vagus nerves. Glutamate (1–30 μmol/L with kynurenate 0.1 mmol/L) concentration dependently inhibited vagal afferent mechanosensitivity. This was mimicked by selective group II and III mGluR agonists but not by a group I agonist. Conversely, a group III mGluR antagonist increased mechanosensitivity to intense stimuli.** ***Conclusions:*** **Both exogenous and endogenous glutamate inhibits mechanosensitivity of vagal afferents. Group II (mGluR2 and 3) and group III mGluR (mGluR4, 6, 7, 8) are novel targets for inhibition of vagal signaling with therapeutic potential in, for example, GERD.**

Glutamate is considered to be the major transmitter involved in signaling from visceral and somatic primary afferents to the central nervous system.[1] These glutamatergic synapses may be subject to modulation by a number of transmitters, including glutamate itself, which act on both ionotropic and metabotropic presynaptic receptors. These receptors are synthesized by the cell bodies of primary afferents and transported to the central endings. The peripheral endings of vagal afferents also possess synaptic specializations and contain the vesicular glutamate transporter VGLUT, indicating that, like their central endings, they are able to release glutamate.[2] We considered the possibility that metabotropic glutamate receptors (mGluR) are transported peripherally, and endogenous or exogenous glutamate may act to influence the function of primary afferent endings.

mGluR exist as 8 subtypes: Group I mGluR (mGluR1 and 5) mostly cause slow depolarization because of activation of phospholipase C,[3] although they can mediate inhibition additionally in some central neurons.[4] Group II (mGluR2 and 3) and group III (mGluR4, 6, 7, and 8) cause slow hyperpolarization via inhibition of adenylate cyclase and altered calcium and potassium currents.[3] The 3 mGluR groups were initially designated based on similarities in their pharmacology. We have shown previously that another inhibitory G-protein-coupled receptor, the $GABA_B$ receptor, is expressed in gastric vagal afferent neurons and inhibits their mechanosensitivity.[5,6] This action of $GABA_B$ receptor agonists translates to inhibition of triggering of transient lower esophageal sphincter (LES) relaxations following gastric distention.[7–10] This in turn results in reduced gastroesophageal acid reflux, with clear therapeutic benefit in patients with reflux disease.[11–15] mGluR have much broader pharmacology and actions, and we considered that they may therefore present greater opportunity for therapeutic intervention than $GABA_B$ receptors.

Abbreviations used in this paper: GERD, gastroesophageal reflux disease; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor.

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In this study, we used electrophysiologic, pharmacologic, molecular, neuroanatomic, and immunohistochemical methods to investigate mGluR associated with vagal afferents from various species. We found that mGluR are widely expressed in cell bodies of nodose ganglia, including gastric afferents, and that they are transported toward the periphery. We also show that activation of group II and III mGluR by exogenous application of agonists reduces mechanical sensitivity of afferent endings, and we provide evidence for a role of endogenous glutamate in the control of mechanoreceptor activation via group III mGluR.

Materials and Methods

RT-PCR Analysis

Total RNA was isolated from nodose ganglia and brain from rat, dog, ferret, and human, using Trizol (Life Technologies, Inc.). One to 2.5 μg total RNA was reverse transcribed at 42°C for 50 minutes in the presence of 0.5 μg/μL oligo (dT)[12–18] primer, 10 × RT buffer (200 mmol/L Tris-HCl, pH 8.4, 500 mmol/L KCl, 25 mmol/L $MgCl_2$), 10 mmol/L DTT, 10 mmol/L of each dNTP, 40 U Recombinant Ribonuclease Inhibitor and 50 U Reverse Transcriptase (SuperScript II, from Life Technologies, Inc., Rockville, MD), in a total volume of 20 μL. The PCR mixture contained 1 μL of the RT reaction, 0.5 μmol/L of each primer, 2 mmol/L of each dNTP, and 1 U Taq polymerase (Applied Biosystems, Foster City, CA). The amplification was carried out according to the following protocol: 95°C for 8 minutes, followed by 25–35 cycles at 95°C for 30 seconds, 56°C for 40 seconds, and 72°C for 40 seconds. PCR-amplified products were separated on 1.5% agarose gels containing ethidium bromide. Brain was used as a positive control, and all reverse-transcription polymerase chain reaction (RT-PCR) products were verified by sequencing. Two negative controls using primers in the absence of cDNA (H_2O and minus RT, respectively) were used for each primer set. Negative controls did not reveal unexpected products. Primer sets were as shown in Table 1.

Retrograde-Labelling Immunohistochemistry

A total of 50 μL of 0.5% cholera toxin B subunit conjugated to FITC (CTB-FITC; List Biological, Campbell, CA) was injected subserosally into the proximal stomach in 5-μL aliquots via a Hamilton microsyringe. After 4 days recovery, ferrets were perfusion fixed, and the nodose ganglia were removed, postfixed, and cryoprotected in 30% sucrose at 4°C for 24–48 hours. Frozen transverse sections at 20 μm were cut serially through the rostrocaudal extent of the nodose ganglia. mGluR immunoreactivity was detected using rabbit polyclonal primary IgG from a number of suppliers (see Table 1) and a goat anti-rabbit secondary IgG conjugated to the red marker Alexa Fluor 546 (Molecular Probes, Eugene, OR). Sections preadsorbed with the immunizing peptide served as negative controls in both control and test tissues. Epifluorescent imaging of mGluR was performed in a number of brain regions of ferret and rodent to confirm the specificity and optimal conditions for the mGluR primary IgGs in the nodose ganglion. Proximal stomach-innervating neurons (FITC labeled green) were counted in 6 to 8 whole ganglion profiles representative of the rostrocaudal extent of each ganglion as previously described.[6]

Table 1. Antibodies and PCR Primers Used for Localization and Detection of mGluR

Antibodies		
Receptor	Sequence	Supplier and catalog number
mGluR1	Residues 1180–1198	Upstate, 06-310
	Residues 1116–1130	Chemicon, AB1595
	Residues 1159–1171	Calbiochem, 445872
mGluR2/3	Residues 853–872	Upstate, 06-676
	Residues 860–872	Chemicon, AB1553
mGluR4	Residues 893–912 within terminal 200 residues	Upstate, 07-765 Zymed, 51-3100
mGluR5	Residues 1153–1171	Upstate, 06-451
	Residues 1159–1171	Chemicon, AB5232
mGluR6	Residues 859–871	Neuromics, RA13105
mGluR7	Residues 899–912	Upstate, 07-239
mGluR8	Residues 889–908	Upstate, 07-174
	Residues 894–908; guinea-pig	Chemicon, AB5362

PCR primers	
Receptor	Sequence and predicted product
mGluR1	Fwd: GTC CCT TCT GAC ACT TTG CAG G Rev: CCA TGT GCC ATG GCA TAG AT, Product size 655 bp
mGluR2	Fwd: ACA TGC ACC GTG CCC TCT Rev: GCT GAG TAG CAG ACA GAG AAG GC Product size 765 bp
mGluR3	Fwd: TCC AAG ATC ATG TTT GTG GTG AA Rev: AAG GGT GTG TTG TTG TGC TTG AT Product size 656 bp
mGluR4	Fwd: TTG CCA ATG AGG ATG ACA TCA G Rev: CAC CTT CCC CTC CTG CTC AT Product size 468 bp
mGluR5	Fwd: AGC CAA TTG ATG GAC GGA AA Rev: TGC AAT ACG GTT GGT CTT GGT Product size 721 bp
mGluR6	Fwd: TTG CCA ATG AGG ATG ACA TCA G Rev: TTT GCG GGT GGA ATC ATC TG Product size 311 bp
mGluR7	Fwd: CGC GTC CTG ACT TTG ATG AA Rev: GAA GCC CCA ATC ACT CCA ACT A Product size 446 bp
mGluR8	Fwd: TGA AGG TTA CAA CTA CCA GGT GGA T Rev: GGC GCT GTG ACA GAT TTC TTC Product size 438 bp

NOTE. All antibodies are used at a dilution of 1:200 and directed to C-termini, and are anti-rat IgG raised in rabbit except for mGluR8 Chemicon.