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Short communication

Transient lower esophageal sphincter relaxations in dogs are inhibited by a metabotropic glutamate receptor 5 antagonist

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Abstract

Transient lower esophageal sphincter relaxation is the major mechanism for gastroesophageal reflux. The present study was initiated to investigate the potential effect of the metabotropic glutamate 5 (mGlu5) receptor antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), on transient lower esophageal sphincter relaxations in the conscious dog. MPEP (1.4–8.7 μmol/kg i.v.) produced a dose-dependent inhibition of transient lower esophageal sphincter relaxations (59±11% inhibition at 8.7 μmol/kg). In addition, there was a reduction of the number of reflux episodes and an increase in latency time to the occurrence of the first transient lower esophageal sphincter relaxation. No effect was seen on basal lower esophageal sphincter pressure or on swallowing.

It is concluded that the mGlu5 receptor antagonist MPEP potently inhibits transient lower esophageal sphincter relaxations and that the mGlu5 receptor is a potential target for treatment of gastroesophageal reflux disease.

Keywords: Glutamate; Lower esophageal sphincter; 2-methyl-6-(phenylethynyl)-pyridine; MPEP; Gastroesophageal reflux

1. Introduction

Transient lower esophageal sphincter relaxations are the major cause of gastroesophageal acid reflux and a potential target mechanism for the treatment of gastroesophageal reflux disease. Transient lower esophageal sphincter relaxations are triggered by gastric distension, leading to an activation of a reflex pathway involving gastric vagal afferents, brainstem centers and inhibitory efferents to the lower esophageal sphincter (Mittal et al., 1995b).

The γ-aminobutyric acid type B ($GABA_B$) receptor agonist baclofen potently reduces the number of transient lower esophageal sphincter relaxations in dogs, ferrets and humans (Blackshaw et al., 1999; Lehmann et al., 1999; Lidums et al., 2000) and importantly, recent data suggest that baclofen reduces reflux and reflux symptoms in gastroesophageal reflux disease patients (Ciccaglione and Marzio, 2003; Vela et al., 2003). Other potential targets involved in the control of transient lower esophageal sphincter relaxations and thus reflux inhibition include cannabinoid, muscarinic and CCK_A receptors (Boulant et al., 1997; Lehmann et al., 2002; Mittal et al., 1995a). However, the side effect profile of ligands for some of these targets makes them less attractive for clinical development.

The metabotropic glutamate (mGlu) receptors belong to the family III of G-protein coupled receptors. Eight different mGlu receptors (mGlu1–mGlu8) have been identified and these can, based on sequence homology, signal transduction mechanisms and pharmacology, be divided into three

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groups (I–III). Binding of glutamate to mGlu1 and mGlu5 receptors (group I) leads to an activation of phospholipase C, while activation of group II and III receptors induces an inhibition of adenylate cyclase (see Conn and Pin, 1997).

Glutamate has been suggested to be a transmitter in gastric vagal afferents and recent data show that most types of mGlu receptors are expressed in the nodose ganglia, containing the nerve cell bodies of gastric vagal afferents, of rat, ferret, dog and human (Chen et al., 2003; Page et al., 2005). Furthermore, retrogradely traced ferret gastric vagal afferents are immunostained by mGlu1–8 receptor antibodies, suggesting the expression of mGlu receptor protein in these gastric vagal afferents (Page et al., 2005). It is therefore of interest to explore the potential role of mGlu receptors in the reflex mediating transient lower esophageal sphincter relaxations and the discovery of specific ligands for some of the mGlu receptors has recently enabled this type of studies. The specific aim of the present study was to investigate the effect of the mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) on transient lower esophageal sphincter relaxations in dog.

2. Materials and methods

2.1. Animals

Adult male and female Labrador retrievers were used in the experiments. Cervical esophagostomies were made and after recovery from surgery, the dogs were accustomed to rest in a Pavlov stand. Before the experiments, the dogs were fasted for approximately 16–18 h, but with free access to water. All procedures were approved by the Ethical Committee for Animal Experiments of the Göteborg region.

2.2. Measurement of transient lower esophageal sphincter relaxations

The method applied for the studies and definitions of motility parameters have been described previously (Lehmann et al., 1999). Briefly, the dogs were intubated with a water-perfused Dentsleeve multilumen assembly for the recording of gastric, lower esophageal sphincter and esophageal pressures. An antimony pH electrode was placed 3 cm above the lower esophageal sphincter for the measurement of reflux episodes and an air-perfused catheter was placed in the hypopharynx to measure swallows.

Transient lower esophageal sphincter relaxations were stimulated by infusion of an acidified liquid nutrient (30 ml/kg) followed by air insufflation (40 ml/min) for the remainder of the experiment. The number of transient lower esophageal sphincter relaxations was measured during a 45 min period starting from the infusion of the liquid. Transient lower esophageal sphincter relaxations were defined as rapid decrease in lower esophageal sphincter pressure (>1 mm Hg/s), to a pressure <2 mm Hg above gastric pressure and a duration >1 s, without any pharyngeal signal <2 s before onset. MPEP was administered as an i.v. bolus dose (0.5 ml/kg) 10 min before start of measurement. For a subset of experiments an i.v. infusion protocol was used, consisting of an initial bolus dose of 2.5 μmol/kg (0.5 ml/kg) followed by 10 μmol/kg (0.5 ml/kg) infused over 55 min.

2.3. Plasma sampling and analysis

Blood samples were taken from a foreleg vein after administration of MPEP (8.7 μmol/kg i.v.). After separation of plasma, the samples were stored at −18 °C until analysis. The analysis of the plasma was performed after protein precipitation with acidic acetonitrile. Chromatographic separation was achieved by gradient elution on a C18 reversed phase column and MPEP was detected by positive ion electrospray tandem mass spectrometry.

2.4. MPEP binding affinity at dog mGluR5

Membranes were prepared from the cerebrum of Beagle dogs by homogenisation and differential centrifugation using the method described for mouse brain membranes (Quéva et al., 2003). Saturation binding experiments with [^{3}H]MPEP (0.5–60 nM, final concentration) in dog brain membranes (60 μg) were performed using a 96-well filtration binding assay (Quéva et al., 2003) with the exception that 0.75% ethanol was omitted from the incubation buffer. Non-specific binding was determined in the presence of 100 μM unlabeled MPEP. Binding affinity (K_d) was determined by fitting the equation $(B_{max}\ x)/(K_d+x)$ to the data using XLfit for Excel (IDBS).

2.5. Drugs

2-methyl-6-(phenylethynyl)-pyridine (MPEP), a specific non-competitive antagonist for the mGlu5 receptor (Gasparini et al., 1999), was synthesized by AstraZeneca R&D, Mölndal, Sweden, according to the procedure of Sonogashira et al. (1975). MPEP was dissolved in 5% ethanol, 40% polyethylene glycol and 55% physiological saline (0.9% NaCl). [^{3}H]MPEP was from Tocris, Bristol, U.K. and was dissolved in 10% ethanol.

2.6. Calculations

Inhibition of transient lower esophageal sphincter relaxations and other parameters were calculated with regard to the mean of five preceding control experiments for each dog. Data are expressed as mean±S.E.M. Statistical analysis was done using paired Student's t-test. $P<0.05$ was regarded as statistically significant. Actual numbers of events were used for statistical analysis.

3. Results

The affinity of MPEP at mGlu5 binding sites in the dog brain was 16±4.6 nM ($n=3$), which is similar to the affinity reported for MPEP at human and rat recombinant mGlu5 receptors (Gasparini et al., 2002; Malherbe et al., 2003).

The average numbers of transient lower esophageal sphincter relaxations and reflux episodes in control experiments were 5.3±0.4 and 4.1±0.3 (five dogs), respectively, which is similar to what has been reported previously (Lehmann et al., 2000, 2002). The mGlu5 receptor antagonist MPEP produced a dose-dependent inhibition of transient lower esophageal sphincter relaxations in the dose range 1.4–8.7 μmol/kg i.v. (Fig 1A; $n=3–4$). The maximal