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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

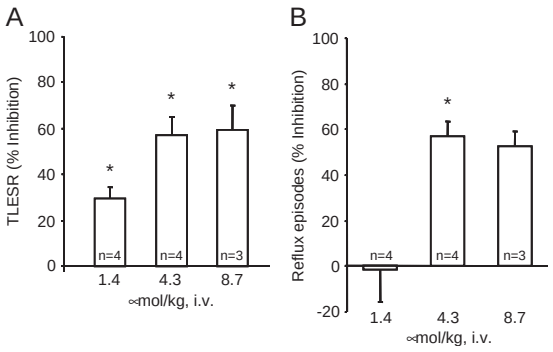

Fig. 1. Inhibitory effect of MPEP 1.4–8.7 μmol/kg (i.v.) on (A) transient lower esophageal sphincter relaxations (TLESR) and (B) gastroesophageal reflux episodes. The measurements started 10 min after MPEP administration and lasted for 45 min. *$P<0.05$.

inhibition of transient lower esophageal sphincter relaxations produced within this dose range was 59±11% (from 5.6±0.2 in control experiments to 2.3±0.7 in MPEP treated dogs). MPEP also induced a significant reduction of the number of reflux episodes at 4.3 μmol/kg (from 4.0±0.8 in control experiments to 1.8±0.5 in MPEP treated dogs), similar in magnitude to the inhibition of transient lower esophageal sphincter relaxations (Fig 1B). At the highest dose, emesis occurred in two out of five dogs and therefore higher bolus doses were not tested. In an attempt to avoid the emetic effect an infusion protocol was adopted, to obtain a slower increase and a more stable plasma concentration of MPEP. An initial bolus dose of 2.5 μmol/kg was followed by a 55 min infusion of 10 μmol/kg. However, also in this case, emesis was observed.

In addition to the effect on transient lower esophageal sphincter relaxations and reflux episodes there was an increase in latency time to the occurrence of the first transient lower esophageal sphincter relaxation in the experiments (78±18% at 4.3 μmol/kg), while no significant effects were seen on basal lower esophageal sphincter pressure, swallowing or esophageal peristalsis (data not shown).

The plasma levels of MPEP after i.v. administration of 8.7 μmol /kg are shown in Fig. 2.

4. Discussion

The present study demonstrates that the mGlu5 receptor antagonist MPEP inhibits the occurrence of transient lower esophageal sphincter relaxations and gastroesophageal reflux in dog, and suggests that the mGlu5 receptor is involved in the mechanism of transient lower esophageal sphincter relaxations. This observation is supported by recent results obtained in the ferret demonstrating that both MPEP and MTEP, another mGlu5 receptor antagonist, reduce the occurrence of transient lower esophageal sphincter relaxations (Frisby et al., 2004). The prolongation of the latency time to the occurrence of the first transient lower esophageal sphincter relaxation is consistent with an elevation of the threshold for initiation of transient lower esophageal sphincter relaxations and this has previously also been shown for $GABA_B$ and cannabinoid receptor agonists (Lehmann et al., 1999, 2002). However, in contrast to $GABA_B$ and cannabinoid receptor agonists, the mGlu5 receptor antagonist did not induce any reduction of the swallowing frequency.

The degree of inhibition of transient lower esophageal sphincter relaxations was similar at the two highest doses of MPEP, suggesting a maximal response close to 60% inhibition, although this could not be further explored due to the emetic effect observed in two out of five dogs at the highest dose and also when using an infusion protocol. However, emesis was only observed in the experimental situation and did not occur in non-instrumented dogs given equal or slightly higher doses of MPEP. This discrepancy may be due to the combined effect of a gastric load and esophageal intubation with the inhibitory effect of MPEP on transient lower esophageal sphincter relaxations, although emesis has not been reported in similar studies for other inhibitory agents (Lehmann et al., 1999, 2002). It is possible that MPEP lowers the threshold level for induction of emesis, but further studies are needed to investigate the involvement of mGlu5 receptor antagonists in the emetic response. No other side effects were observed with MPEP at the doses given.

The site of action of MPEP mediating inhibition of transient lower esophageal sphincter relaxations is not known at present. Immunohistochemical studies have shown the occurrence of mGlu5 receptors in the enteric nervous system (Liu and Kirchgessner, 2000). However, an effect on enteric neurons would be expected to produce a graded increase in lower esophageal sphincter pressure and/or incomplete transient lower esophageal sphincter relaxations. As no such effects were noted it is not likely that the effect on transient lower esophageal sphincter relaxations is mediated via enteric neurons. Expression of mGlu5 receptors in the nodose ganglion has been demonstrated in several species including dog, ferret and human, and vagal ligation studies in ferret show a proximal accumulation of mGlu5 receptor immunoreactivity, suggesting a possible localization to gastric vagal afferent peripheral terminals (Page et al., 2005). In addition, mGlu5 receptors are expressed in the nucleus tractus solitarius (Hoang and Hay, 2001), indicating the possibility that mGlu5 receptors are involved in

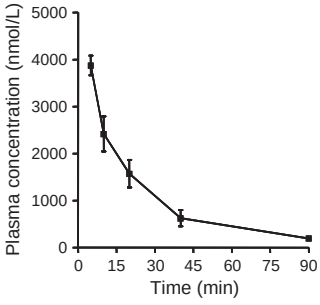

Fig. 2. Plasma concentration of MPEP in dog after i.v. administration of 8.7 μmol/kg ($n=3$). The experimental period lasted from 10 to 55 min after administration.

modulation of afferent input into the brain stem. Clearly further studies are needed before any conclusions can be drawn regarding a central and/or peripheral mechanism for mGlu5 receptors in inhibition of transient lower esophageal sphincter relaxations.

It can be concluded that the mGlu5 receptor antagonist MPEP has an inhibitory effect on transient lower esophageal sphincter relaxations and gastroesophageal reflux in dog and that the mGlu5 receptor is a potential target for treatment of gastroesophageal reflux disease.

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References

Blackshaw, L.A., Staunton, E., Lehmann, A., Dent, J., 1999. Inhibition of transient LES relaxations and reflux in ferrets by GABA receptor agonists. Am. J. Physiol. 277, G867–G874.

Boulant, J., Mathieu, S., D'Amato, M., Abergel, A., Dapoigny, M., Bommelaer, G., 1997. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 40, 575–581.

Chen, C.Y., Ling Eh, E.H., Horowitz, J.M., Bonham, A.C., 2003. Synaptic transmission in nucleus tractus solitarius is depressed by group II and III but not group I presynaptic metabotropic glutamate receptors. J. Physiol. 538, 773–786.

Ciccaglione, A.F., Marzio, L., 2003. Effect of acute and chronic administration of the GABAB agonist baclofen on 24 hour pH metry and symptoms in control subjects and in patients with gastro-oesophageal reflux disease. Gut 52, 464–470.

Conn, P.J., Pin, J.-P., 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu. Rev. Pharmacol. Toxicol. 37, 205–237.

Frisby, C., Mattsson, J., Jensen, J., Lehmann, A., Dent, J., Blackshaw, L.A., 2004. Involvement of group-I metabotropic glutamate receptors in the mechanism of transient LES relaxation in conscious ferret. Gastroenterology 126 (Suppl. 2), A-56.

Gasparini, F., Lingenhohl, K., Stoehr, N., Flor, P.J., Heinrich, M., Vranesic, I., Biollaz, M., Allgeier, H., Heckendorn, R., Urwyler, S., Varney, M.A., Johnson, E.C., Hess, S.D., Rao, S.P., Sacaan, A.I., Santori, E.M., Velicelebi, G., Kuhn, R., 1999. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38, 1493–1503.

Gasparini, F., Andres, H., Flor, P.J., Heinrich, M., Inderbitzin, W., Lingenhohl, K., Muller, H., Munk, V.C., Omilusik, K., Stierlin, C., Stoehr, N., Vranesic, I., Kuhn, R., 2002. [(3)H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5. Bioorg. Med. Chem. Lett. 12, 407–409.

Hoang, C.J., Hay, M., 2001. Expression of metabotropic glutamate receptors in nodose ganglia and the nucleus of the solitary tract. Am. J. Physiol. 281, H457–H462.

Lehmann, A., Antonsson, M., Bremner-Danielsen, M., Flärdh, M., Hansson-Brändén, L., Kärrberg, L., 1999. Activation of the $GABA_B$ receptor inhibits transient lower esophageal sphincter relaxations in dogs. Gastroenterology 117, 1147–1154.

Lehmann, A., Hansson-Brändén, L., Kärrberg, L., 2000. Effects of repeated administration of baclofen on transient lower esophageal sphincter relaxation in the dog. Eur. J. Pharmacol. 404, 163–167.

Lehmann, A., Blackshaw, L.A., Brändén, L., Carlsson, A., Jensen, J., Smid, S.D., 2002. Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxations and reflux in dogs. Gastroenterology 123, 1129–1134.

Lidums, I., Lehmann, A., Checklin, H., Dent, J., Holloway, R.H., 2000. Control of transient lower esophageal sphincter relaxations and reflux by the $GABA_B$ agonist baclofen in normal subjects. Gastroenterology 118, 7–13.

Liu, M.T., Kirchgessner, A.L., 2000. Agonist- and reflex-evoked internalization of metabotropic glutamate receptor 5 in enteric neurons. J. Neurosci. 20, 3200–3205.

Malherbe, P., Kratochwil, N., Zenner, M.-T., Piussi, J., Diener, C., Kratzeisen, C., Fischer, C., Porter, R.H.P., 2003. Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine. Mol. Pharmacol. 64, 823–832.

Mittal, R.K., Holloway, R., Dent, J., 1995a. Effect of atropine on the frequency of reflux and transient lower esophageal sphincter relaxation in normal subjects. Gastroenterology 109, 1547–1554.

Mittal, R.K., Holloway, R.H., Penagini, R., Blackshaw, L.A., Dent, J., 1995b. Transient lower esophageal sphincter relaxation. Gastroenterology 109, 601–610.

Page, A.J., Young, R.L., Martin, C.M., Umaerus, M., O'Donell, T.A., Cooper, N.J., Coldwell, J.R., Hulander, M., Mattsson, J.P., Lehmann, A., Blackshaw, L.A., 2005. Metabotropic glutamate receptors inhibit mechanosensitivity in vagal sensory neurons. Gastroenterology 128, 402–410.

Quéva, C., Bremner-Danielsen, M., Edlund, A., Ekstrand, A.J., Elg, S., Erickson, S., Johansson, T., Lehmann, A., Mattsson, J.P., 2003. Effects of GABA agonists on body temperature regulation in GABA(B(1))−/− mice. Br. J. Pharmacol. 140, 315–322.

Sonogashira, K., Tohda, Y., Hagihara, N., 1975. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. Tetrahedron Lett. 50, 4467–4470.

Vela, M.F., Tutuian, R., Katz, P.O., Castell, D.O., 2003. Baclofen decreases acid and non-acid post-prandial gastro-oesophageal reflux measured by combined multichannel intraluminal impedance and pH. Aliment. Pharmacol. Ther. 17, 243–251.