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Differential Effects of TRPV1 Antagonists in Acid-induced Excitation of Esophageal Vagal Afferent Fibers of Rats

S. Peles¹, B. K. Medda¹, Zhihong Zhang¹, B. Banerjee¹, A. Lehmann², R. Shaker¹, and J.N. Sengupta¹

¹ Division of Gastroenterology & Hepatology, Medical College of Wisconsin, Milwaukee, Wisconsin

² Integrative Pharmacology, AstraZeneca R&D Mölndal, Mölndal, Sweden

Abstract

Gastro-esophageal acid reflux can stimulate esophageal vagal sensory afferents by activating proton-sensitive ion channel transient receptor vanilloid one (TRPV1). The objective of this study was to investigate the response characteristics of vagal afferent fibers of rats to acid (0.1N HCl) and capsaicin (CAP) following esophagitis and differential effects of two classes of TRPV1 antagonists on responses of vagal afferent fibers. The chronic reflux was induced by ligating the fundus of the stomach and partial constriction of pylorus. Extracellular single fiber recordings were made from the cervical vagal afferent fibers from naïve control and fundus-ligated (FL) esophagitis rats. Innervations of fibers were identified to esophageal distension (ED) and subsequently tested to CAP and acid before and after injection of TRPV1 antagonist JYL1421 or AMG9810 (10 μmol/kg, i.v.). Seventy-five vagal afferent fibers from 70 rats were identified to ED. Intra-esophageal CAP (0.1 ml of 1 mg/ml) excited 39.5% (17/43, 5/22 from naïve and 12/21 from FL rats) fibers. In contrast, intravenous (i.v.) injection of CAP (0.03–0.3 μmol/kg) dose-dependently excited 72% (42/58) fibers. Responses to CAP were significantly greater for fibers from FL rats (n=32) than naïve rats (n=25). TRPV1 antagonists JYL1421 and AMG9810 (10 μmol/kg) significantly blocked response to CAP. Intra-esophageal acid infusion stimulated 5/17 (29.4%) fibers from naïve rats and 12/28 (42%) from FL rats. Effect of acid was significantly blocked by AMG9810, but not by JYL1421. Results indicate that following esophagitis the number of fibers responsive to CAP and acid is greater than non-inflamed esophagus, which may contribute to esophageal hypersensitivity. Acid-induced excitation of vagal sensory afferents can be differentially attenuated by different classes of TRPV1 antagonists. Therefore, TRPV1 antagonists play a key role in attenuation of hypersensitivity following reflux-induced esophagitis. The use of TRPV1 antagonists could be an alternative to the traditional symptoms based treatment of chronic acid reflux and esophageal hypersensitivity.

Keywords

Vagus; esophagus; hypersensitivity; capsaicin; esophagitis; acid reflux

Address for correspondence: Jyoti N. Sengupta, Division of Gastroenterology and Hepatology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, Tel: 414-456-4011, Fax: 414-456-6361, Email: E-mail: sengupta@mcw.edu.

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Introduction

Gastroesophageal reflux disease (GERD) is a common disorder often reported as severe heartburn and pain in the upper thoracic chest. It has been reported that prolonged acid in the esophagus triggers esophageal hypersensitivity (Drewes et al., 2002, 2003, Hu et al., 2000, Pedersen et al., 2004, Sarkar et al., 2000, 2001, 2004). Several mechanisms have been proposed to account for the activation of primary sensory afferents by acid including activation of acid-sensing ion channels (ASICs) (Chen et al. 1998, Waldmann et al 1997a,b) and the capsaicin (CAP)-sensitive transient receptor potential vanilloid one (TRPV1) channel (Caterina et al 1997, Jordt et al 2000, Kress et al 1996, Martenson et al 1994, Petersen & LaMotte 1993, Tominaga et al 1998). Initially it was thought that TRPV1 was associated with somatic thermal hyperalgesia in tissue inflammation (Caterina & Julius 2001), however recent studies suggest that TRPV1 plays a significant role in visceral pain and hypersensitivity associated with tissue inflammation (Apostolidis et al., 2004, Dinis et al., 2004, Fujino et al., 2004, Matthews et al., 2004, Schicho et al., 2004, Yiangou et al., 2001). In humans, the expression of TRPV1 receptors in the lamina propria increases in inflammatory bowel disease and esophagitis (Matthews et al 2004, Yiangou et al., 2001). Similarly, in experimental animals TRPV1 expression increases in the dorsal root ganglia (DRG) following acid exposure to gastric mucosa and esophagus (Schicho et al. 2004, Banerjee et al., 2007). In the rat model of cystitis, TRPV1 plays an important role in bladder hyperreflexia, which can be significantly attenuated by blocking the TRPV1 channels (Dinis et al., 2004). It has been shown that hyperreflexia in cystitis occurs due to activation of TRPV1 channel by inflammation-induced release of endovanilloids anandamide that acts as an endogenous ligand for TRPV1 channels (Dinis et al., 2004).

Although sparse relative to dorsal root ganglia (DRG), TRPV1 channels are present in the nodose ganglia (NG) (Michael & Priestly 1999, Patterson et al 2003, Ward et al 2003, Banerjee et al., 2007) and CAP activates vagal afferent fiber innervating esophagus and stomach (Blackshaw et al, 2000,). Our recent study has shown that reflux-induced chronic esophagitis results in significant increase in TRPV1 expression in NG (Banerjee et al., 2007). Similarly, in TRPV1 deficient mice reflux promoting surgery induces significantly less mucosal injury and inflammation than the wild type mice and pretreatment with TRPV1 antagonist capsazepine in wild type mice significantly inhibits esophagitis. These results suggest that TRPV1 channel is closely associated with tissue inflammation.

TRPV1 receptors can contribute to esophageal inflammation through neurogenic inflammation and acid activated secretion of pro-inflammatory cytokines (IL-6, IL-8) from mucosal epithelial cells. In neurogenic inflammation, acid stimulates the sensory neurons via the activation of TRPV1 receptor to release neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) resulting in vasodilatation and plasma extravasation. TRPV1 is constitutively present in epithelial cells including bronchial, gastric, oral, urinary bladder, prostate and cornea (Agopyan et al., 2003, Veronesi et al., 1999, Birder et al., 2001, Kato et al., 2003, Kido et al., 2003, Inoue et al., 2002, Southall et al., 2003, Sanchez et al., 2005, Zhang et al., 2007). Therefore, activation of epithelial TRPV1 receptors by acid results in release of cytokines to produce mucosal injury. Considering the sensitivity of TRPV1 receptors to acid, its presence and increase in expression during esophagitis it is conceivable that TRPV1 receptor is possibly associated with acid-induced altered sensation of the esophagus. However, this has not been investigated systematically, especially in chronic esophagitis. We hypothesize that following esophagitis vagal afferent fibers will be more sensitive to CAP and acid, which can be attenuated by TRPV1 antagonists. Objectives of the present study are: (1) to characterize responses of esophageal distension-sensitive vagal afferent fibers to acid and CAP in naïve control and FL rats having chronic esophagitis, (2) to evaluate the contribution of the TRPV1 receptor in sensitization of vagal afferent fibers to acid and (3) to study the effects of the

selective TRPV1 antagonists in attenuation of responses esophageal distension(ED)-sensitive vagal afferent fibers to CAP and acid (0.1N HCl).

Methods

Experiments were performed on 70 male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 400–500g. The Institutional Animal Care and Use Committee (IACUC) of the Medical College of Wisconsin approved all experimental procedure in accordance to the guidelines of the International Association of Study of Pain. Rats were deprived of food 16–18 hours prior to the surgical procedure, but water was freely available.

Reflux promoting surgery

For inducing gastric reflux we followed the method as previously described (Omura et al., 1999, Banerjee et al., 2007). Briefly, rats were anesthetized using sodium pentobarbital (50 mg/kg. i.p.; Nembutal; Abbott Laboratories, USA) and the upper abdomen was opened by giving a midline incision. The stomach was retracted with a blunt non-serrated artery clamp and a ligation was placed along the limiting ridge between the fundus and glandular part of the stomach. In addition, an 18Fr Nalaton catheter ring (2mm in thickness, ID: 5–6mm, OD: 8–9mm) was placed around the pylorus to restrict emptying of gastric content into the duodenum. The wound was closed by suturing muscle followed by skin. Rats received 0.2ml of 2.27% Baytril antibacterial injectable solution (Baytril, Bayer HealthCare LLC, USA) and Carprofen (5mg/kg, im, Rimadyl, Pfizer, USA) seven days post-surgery to eliminate infection and pain, respectively. Experiments were performed 7 days following surgery. The tissue inflammation in the rat was examined after electrophysiology recording. Rats were euthanized as described in the following section, thorax was opened and the entire length of the esophagus was removed. The lumen was opened longitudinally and macroscopic changes such as mucosal erosion, deep ulcer and thickening of the muscle wall were examined. Some esophageal tissues, but not all, were stored in 4% paraformaldehyde for eosin and hematoxylin staining and subsequent microscopic evaluation.

Electrophysiology experiments—Rats were anesthetized initially with sodium pentobarbital (50mg/kg. ip; Nembutal; Abbott Laboratories, USA) and maintained with a constant infusion of sodium pentobarbital (5–10 mg/kg/h, iv). Catheters (PE-50, Beckton & Dickenson) were inserted into the left carotid artery and left femoral vein to monitor blood pressure and for drug infusion, respectively. The trachea was intubated and the rat was ventilated with room air (~ 60 strokes/min; Kent ventilator, model-3000; Kent Scientific, Torrington, CT, USA) after paralyzing it with muscle relaxant gallamine triethiodide (10 mg/kg. i.v.). Supplemental doses of gallamine triethiodide (1 mg/kg/hr, i.v.) were administered to maintain the paralysis throughout experiment. At the end of experiment, rats were euthanized by injecting 0.25ml/kg of Beuthanasia-D (390 mg pentobarbital + 50 mg phenytoin sodium + 2% benzyl alcohol; Schering-Plough, USA).

The right cervical vagus nerve was exposed by a midline incision in the neck and removal of the sternocleidomastoid, sternohyoid, and omohyoid muscles. A small pool was made by retracting the neck skin using silk sutures tied to a stereotaxic frame. The pool was filled with warm mineral oil, and the right vagus nerve was separated from the carotid artery, and sympathetic nerve. The nerve was transected below the entry of superior laryngeal nerve and placed on a black micro-base plate. The perineural sheath was gently removed and a small filament of nerve axon was teased out to obtain single-unit recordings. For differential recordings, fibers were draped on one arm of the bipolar platinum electrode while the other arm of the electrode was connected to a strand of connective tissue. Action potentials were displayed continuously on a digital storage oscilloscope (model-VC7525, Hitachi, Japan) after

amplification through the amplifier (model-3000: A-M Systems, Inc. USA). The analog delay dual window discriminator (DDS-1; BAK Instrument, USA) was used to discriminate the action potentials of single waveform and recorded on-line using the Spike2 data acquisition system (CED 1401plus, Cambridge Electronic Design, Cambridge, UK). The firing frequency of the nerve, represented as peri-stimulus time histograms (PSTH, 1sec bin width), action potentials, esophageal distension pressure, and blood pressure were continuously displayed on-line and stored on a PC-based computer (Dell Dimension 8400, USA). All nerve action potentials were further analyzed post-experiment using Spike2 wave mark analysis (Spike2, version 4.01 software).

Experimental protocol—Distension-sensitive vagal afferents were identified by esophageal distension (ED, 40mmHg, 5–10s) using a flaccid polyethylene balloon (1cm in length, 2cm² when inflated) connected to an isobaric distension device. Once the fiber was identified, a stimulus-response function (SRF) to graded ED (10, 20, 30, 40, and 60mmHg, 30sec with 3min inter-stimulus interval) was constructed. In initial experiments, influences of TRPV1 antagonist JYL1421 or AMG9810 on mechanotransduction properties of afferent fibers were tested by constructing a SRF to graded ED before and two minutes after injection of antagonist (10 µmol/kg, i.v.).

In a different set of experiments, naïve control and FL esophagitis rats received intra-esophageal saline infusion (0.1ml @ 0.1ml/min) followed by CAP (0.1ml of 2.5µM@0.1ml/min) to test the sensitivity to CAP. In similar manner, the effect of intra-esophageal acid (0.1ml of 0.1N HCl) infusion was tested before and 5 minutes after application of TRPV1 antagonist. In preliminary test, we have found that intraluminal acid and CAP at shorter intervals (10–15 minutes), results in complete desensitization of response to acid or CAP and to esophageal distension (ED). Therefore, in subsequent experiments intraluminal acid or CAP was injected after 30 minutes interval to achieve reproducible response. Similarly, intravenous (i.v.) CAP at shorter intervals (10–15 minutes) produces desensitization of response to subsequent higher dose of the drug. Therefore, in order to achieve a dose-dependant response CAP was injected after 30 minutes interval. The blocking effect of TRPV1 antagonist was tested by injecting (10 µmol/kg, i.v.) the drug two minutes prior to application of acid or CAP.

Drugs—CAP (8 Methyl-n-vanillyl-6-nonenamide, Sigma, USA) was prepared by dissolving 1mg in 0.1 ml of 100% ethanol and 0.1 ml of Tween 80 (polyoxyethylenesorbitan monoooleate, Sigma, St. Louis USA). The solution was then made 1mg/ml by adding 0.8 ml of distilled water. JYL1421 (synthesized by AstraZeneca, Sweden) and AMG9810 (Tocris, MO, USA) were prepared similar fashion by dissolving 10mg of the compound in 0.2 ml of 100% ethanol and 0.2 ml of Tween 80 followed by 0.6 ml of distilled water to make 10mg/ml solution.

Data analysis and Statistics—For electrophysiology experiment the mean firing frequency (impulses/s) of the fiber to ED was calculated by subtracting it from the mean firing frequency of 60 sec ongoing spontaneous activity. Data are expressed as mean ± SEM and were analyzed at each distension pressure using one-way repeated measures ANOVA. The response to CAP and acid were calculated by subtracting the mean firing frequency of 60 sec baseline ongoing activity before application of the chemical from the mean firing frequency of 30 sec following the application of chemical. The difference between the CAP or acid response before and after application of TRPV1 antagonists was analyzed using a Mann-Whitney Rank Sum Test. A 'p' value <0.05 was considered for significance.

Results

Tissue inflammation

The inflammation was detected in FL rats, which was more intense in the distal and mid-thoracic esophagus. Tissues exhibited progressive thickening of the stratified squamous epithelium, but there was no evidence of columnar epithelium in the distal esophagus. Thus, it indicates that the stomach epithelium did not infiltrate the esophagus. A progressive hyperplasia of the lamina propria and submucosa was seen along with the presence of neutrophils, eosinophils and basal cells.

Mechanosensitivity of vagal afferent fibers

Seventy-five vagal afferent fibers from 70 rats were identified as esophageal distension (ED)-sensitive fibers exhibiting at least 50% increase in firing rate to 40mmHg ED. All fibers exhibited spontaneous firing with or without a balloon in the esophagus. There was no significant difference in mean spontaneous firing between the naïve control (non-inflamed) and FL groups (9.8 ± 2.2 , $n=22$, vs 11.2 ± 2.4 imp/s, $p = 0.626$). Typically, the firing frequencies of all ED-sensitive fibers increased linearly to increasing intensity of distension up to the distending pressure of 40mmHg (fig 1A). However, at greater intensity (60mmHg) the mean response was not significantly greater than that at 40mmHg. Fibers from FL rats appeared to have higher response compared to naïve rats, but the difference was not statistically significant ($p = 0.533$) (fig 1B).

Influence of TRPV1 antagonist JYL1421 and AMG9810 on mechanotransduction

To test whether TRPV1 antagonists JYL1421 and AMG9810 influence the spontaneous firing and mechanotransduction in 10 ED-sensitive afferent fibers from naïve rats stimulus-response functions (SRFs) to graded distension were constructed prior to and 2 minutes after the injection of the antagonist (10 μ mol/kg, i.v.). Both antagonists had no effect on the spontaneous firing of these fibers. The mean spontaneous firing before and after the application of JYL1421 was 5.3 ± 1.2 imp/s ($n=6$) and 5.2 ± 2.5 imp/s, respectively. Similarly, the mean spontaneous firing prior to AMG9810 injection was 7.61 ± 1.41 imp/s ($n=4$) and following drug injection it was 8.44 ± 1.86 imp/s. Both antagonists did not influence SRFs of these fibers to graded ED (figs. 1C and 1D), suggesting that TRPV1 antagonists do not affect the mechanotransduction property of esophageal vagal afferent fibers.

Effect of Capsaicin (CAP)

Intraluminal infusion of CAP (0.1ml of 1mg/ml) excited 17/43 fibers. In fundus ligated (FL) rats, 57% (12/21) fibers responded to intraluminal CAP compared to 22% (5/22 fibers) in naïve control rats. In contrast, intravenous (i.v.) injection of CAP (0.03, 0.1, and 0.3 μ mol/kg) dose-dependently excited 72% (42/58) fibers from two groups of rats (naïve $n=14$, FL $n=28$). In naïve rats, CAP produced dose-dependent excitation that lasted for a brief period (<60s) (fig 3A), whereas in majority of fibers from FL rats excitation to CAP lasted ~ 60–120s (fig 2A). Both JYL1421 and AMG 9810 (10 μ mol/kg i.v.) significantly blocked CAP response in all tested doses (figs. 2 and 3). Figure 4 summarizes data of CAP-induced excitation and effects of JYL1421 and AMG 9810 in naïve and FL rats. Responses of fibers from FL rats CAP at 0.03, and 0.1 μ mol/kg were significantly higher than that of naïve rats (fig 4, $p<0.05$ vs naïve).

Effect of 0.1N HCl

The effect of intra-esophageal acid (0.1N HCl, 0.1ml @ 0.1ml/min) was tested on 45 ED-sensitive afferent fibers (17 from naïve rats and 28 from FL rats). In naïve rats, 5/17 (29.4%) fibers responded to acid. However, in FL rats a greater number (12/28) of fibers responded to intraluminal acid. There was no significant difference in the intensity of response between

naïve and FL rats (fig 5C). Fibers exhibited an increase in firing to acid that progressed to a sustained elevated firing greater than baseline (fig 5A, B and 6C). JYL1421 (10 μ mol/kg i.v.) did not attenuate acid-induced activation of fibers in any group (fig 5C). Effect of AMG9810 was tested on responses of four fibers to acid from FL rats. In all fibers, AMG 9810 (10 μ mol/kg, i.v.) significantly inhibited the effect of acid (figs 6 and 7, $p < 0.05$ vs pre-AMG9810 acid response).

Discussion

Summary of results

Objective of the present study was to characterize response properties of ED-sensitive vagal afferent fibers to acid and CAP in naïve non-inflamed and fundus ligated (FL) rats having chronic esophagitis. The study was also directed to evaluate the contribution of different classes of TRPV1 receptor antagonists in modulation of responses of sensitized vagal afferent fibers to acid and CAP. The study demonstrates that chronic reflux of gastric contents following fundus ligation produces esophageal tissue inflammation in rats. Overall, CAP can excite only 39% (17/43) ED-sensitive afferent fibers when injected into the lumen of the esophagus. In contrast, significantly a greater number (72%, 42/58) of fibers were excited when the CAP was injected intravenously. Of 17 fibers that responded to intra-esophageal CAP infusion, a greater proportion (57%, 12/21) of fibers were from FL esophagitis rats, when only 22% (5/22) were from naïve rats. The dose-response relationship to intravenous injection of CAP revealed significantly higher intensity of firing of fibers from FL rats than naïve rats. Intra-esophageal acid activated about 29% (5/17) ED-sensitive fibers from naïve rats, whereas in FL rats 42% (12/28) fibers were sensitive to acid. Although TRPV1 receptor antagonist JYL1421 and AMG9810 significantly blocked the effect of CAP, only AMG9810 attenuated the effect of acid-induced excitation of fibers. This result suggests that excitation by acid is differentially blocked by different classes of TRPV1 antagonists.

Mechanosensitive properties

Vagal afferent fibers innervating the muscle generally respond to intraluminal distension (Andrew 1956, Blackshaw et al., 2000, Clerc and Mei 1983, Dong et al., 2001, Satchell 1984, Sekizawa et al., 1999, Sengupta et al., 1989, 1990, 1992, Yu et al., 2005, Zagorodnyuk et al., 2000, 2003,). In vitro electrophysiology recording antegrade labeling of the recorded vagal afferent fiber has revealed that the distension-sensitive afferent fibers in the esophagus are primarily intraganglionic laminar endings (IGLEs), which typically exhibit slowly-adapting response to isobaric distension (Zagorodnyuk et al., 2000). The tonic firing during the period of distension undergoes a decrease in firing frequency below the resting frequency immediately after termination of distension (fig 1A). This decline in firing is due to sudden loss of muscle tone and firing progressively regains with recovery of muscle tone. This pattern of response is not dependent on the type of muscle (striated or smooth) the fiber innervates. Vagal distension-sensitive afferent fibers often exhibit a typical character of saturation of firing frequency at greater intensities of distension. This phenomenon has been documented in opossum (Sengupta et al., 1989, 1992), ferret (Blackshaw et al., 2000), dog (Satchell 1984) and guinea-pig (Yu et al., 2005). In the present study about 80% of muscle afferent fibers from naïve and FL esophagitis rats exhibited a saturation of response to distending pressure >40mmHg (fig 1B).

Chemosensitive properties of vagal afferent fibers

Majority of distension-sensitive muscle afferents are multimodal in nature that respond to chemical stimuli (e.g., HCl, CAP, ATP, bradykinin, GABA_B-receptor agonists). About 15–30% of muscle afferent fibers from different species exhibit excitation to intraluminal acid perfusion (Sekizawa et al., 1999, Page and Blackshaw 1998, Medda et al., 2005). In the present

study, 29% of ED-sensitive vagal afferent fibers responded to intraluminal infusion of acid. It is generally thought that due to the deep location of the terminals in the muscle and tight mucosal barrier intraluminal acid is inaccessible to excite these afferent fibers. However, in the mouse acid failed to activate stretch-sensitive muscle afferent fibers despite the thin musculature and greater accessibility of luminal contents to the nerve endings (Page et al., 2001). In contrast, relatively greater proportion of muscle afferent fibers respond to other chemicals (e.g., α , β -methylene ATP, 5-HT, bile and CAP) when applied topically on the mucosal surface in this species (Blackshaw et al., 2000, Page and Blackshaw 1998, Page et al., 2001). In the present study, acid- and CAP-sensitive afferent fibers could be similar to tension/mucosal (T/M) fibers of ferrets that respond to mechanical stretch, mucosal probing and chemicals (Page and Blackshaw 1998). Although there is no morphological evidence, it is speculated that T/M afferent fibers have axon collaterals innervating muscle and mucosa. Our study also demonstrates that in FL esophagitis relatively more fibers respond to intra-esophageal acid infusion. This could be due to sensitization of greater number T/M fibers to chemicals and/or erosive destruction of mucosal barrier that allows acid to diffuse into the muscle layer.

The distension-sensitive muscle afferents are sensitive to CAP (Blackshaw et al., 2000, Page and Blackshaw 1998). Like acid, intra-esophageal CAP perfusion did not excite many ED-sensitive afferent fibers in naïve rats. This result is very similar to *in vivo* study in ferrets where intraluminal application of CAP activated only 20% (2/10) of esophageal distension-sensitive afferent fibers and 100% (1/1) of mucosal afferent fibers (Blackshaw et al., 2000). In the present study following esophagitis more fibers responded to intra-esophageal infusion of CAP. Intravenous CAP stimulated significantly greater number of fibers compared to intra-esophageal perfusion. Many fibers that did not respond to intraluminal CAP responded to intravenous injection, suggesting that mucosal barrier prevents the drug to excite the nerve terminals. It is very likely that CAP can more easily diffuse through the capillaries to excite the ED-sensitive muscle afferent fibers. It is also possible that many of these fibers have axon collaterals in close proximity of the capillaries and therefore CAP has relatively easy access to excite these terminals. Since there is no morphological data available at this time, this notion would be merely speculative. Dose-response functions of CAP revealed that fibers from FL esophagitis rats responded significantly higher than that of naïve rats. This is possibly due to over expression of TRPV1 receptors in vagal afferents fibers following esophagitis, which we have documented in our recent study (Banerjee et al., 2007).

Effect of TRPV1 antagonist on response to acid

The objective of the present study was to test whether TRPV1 antagonist can prevent acid-induced excitation of vagal afferent fibers. The result shows that although JYL1421 significantly blocks the excitatory effect of CAP, it fails to block the effect of acid. In the guinea-pig trachea, rapidly adapting nodose A δ - and C-fibers exhibiting transient response to acid (citric acid) is not affected by TRPV1 antagonist capsazepine, whereas slowly adapting acid-sensitive C-fibers can be effectively blocked by capsazepine and iodo-resiniferatoxin (Kollarik and Undem 2002). It has been proposed that the slowly adapting response to acid is due to activation of proton-gated TRPV1 channel and therefore can be blocked by TRPV1 antagonist. The transient response to acid in presence of TRPV1 antagonist is possibly due to activation of acid-sensing ion channels (ASICs), since the pattern of response follows the characteristics of ASICs activation (Kollarik and Undem 2002). Although the majority of acid-sensitive vagal afferent fibers (mucosal and muscle) in the GI tract exhibit slowly adapting character to acid similar to tracheal slowly adapting nodose C-fibers (Blackshaw et al., 2000, Page and Blackshaw 1998, Medda et al., 2006), the response cannot be blocked by the TRPV1 antagonist JYL1421. However, AMG 9810 significantly blocked the acid-induced excitation of vagal afferent fibers. This differential result can be explained on the basis of two major factors. First, it has been shown that TRPV1 channels of human and guinea-pig are different

from rat TRPV1 channels. For example, capsaizepine blocks proton-induced activation of human and guinea-pig TRPV1 receptors, but not in rats (McIntyre et al., 2001, Savigde et al., 2001). Similarly, SB-366791, which has similar molecular structure like capsaizepine blocks CAP, but not proton-induced activation of TRPV1 channel in rats (Gavva et al., 2005b). Our findings with JYL1421 are consistent with recent reports.

Secondly, the activity of an antagonist is dependent on the nature of its binding to the 'vanilloid binding pocket' to produce a conformational change (i.e., allosteric inhibition) of the channel pore. It has been recently reported that although all TRPV1 antagonists are competitive antagonist of CAP and bind to vanilloid binding pocket (i.e., the transmembrane 3/4 region of the TRPV1 receptor of rat), not all antagonists produce allosteric inhibition to close the channel pore between TM5 and TM6 (Gavva et al., 2004, 2005a, b). The compound that binds with the binding pocket and blocks the proton-activated opening of the pore is designated as 'Class A' compound (e.g., AMG6880, AMG9810). The compound that binds to the site, but does not produce a conformational change to close the channel pore is designated as 'Class B' compound (e.g., capsaizepine, AMG0610, SB-366791). Therefore, our result clearly demonstrates the differential effect of two classes of TRPV1 antagonists and JYL1421 appears to class B antagonist.

In our recent study, pre-emptive JYL1421 treatment for seven days significantly prevented colonic inflammation and attenuated colonic pain induced acidic saline (pH 5.0) (Miranda et al., 2007). However, the attenuation of acid-induced visceral pain was possibly not due to blockade of proton-induced channel activation by JYL1421, but due to significant downregulation of the channel in the thoraco-lumbar (T13-L1) and lumbo-sacral (L6-S1) DRGs following chronic JYL1421 treatment. In contrast to pre-emptive treatment, when JYL1421 was injected after the onset of inflammation (i.e., post-emptive) rats exhibited pain to intracolonic acidic saline and there was no downregulation of TRPV1 channels in DRGs of these rats.

TRPV1 and visceral hyperalgesia

A large number of studies have documented the role of TRPV1 receptors in somatic pain, especially its contribution in somatic thermal hyperalgesia (Caterina et al., 1997, Lee and Caterina 2005, Pogatzki-Zahn et al., 2005, Roberts and Connors 2006). The emerging literature indicates that TRPV1 is possibly playing a key role in visceral pain under pathological conditions. It is well established that endovanilloids including endocannabinoid (e.g., anandamide), lipoxygenase derivatives (e.g., 12-HPETESs, 15-HEPTES and LTB4) and long-chain unsaturated n-acyl dopamines released during inflammation can potently activate TRPV1 channels (Petrocellis and Marzo 2005, Petrocellis et al., 2004, Ross 2003). A recent study using an animal model of cystitis has clearly shown that anandamide released after bladder inflammation produces hyperreflexia, which can be blocked by TRPV1 antagonist (Dinis et al., 2004). A clinical study in humans has documented that in patient with rectal hypersensitivity and fecal urgency TRPV1-ir in sensory nerve fibers innervating the muscle, submucosal, and mucosal layers is markedly higher compared to normal individual (Chan et al., 2003). This increase in TRPV1-ir closely correlates with the decrease in thresholds for rectal heat and distension pressure.

It is well recognized that frequent acid exposure can lead to esophagitis and heavy acid burden often produces esophageal hypersensitivity. While healing of erosive esophagitis can be controlled by the use of proton pump inhibitors (PPI), therapy for non-erosive reflux disease (NERD) still remains challenging. TRPV1 antagonist that can potentially block the acid and endovanilloid-induced tissue injury may become therapeutically important to prevent esophageal hypersensitivity and tissue inflammation. Our recent study demonstrates that preemptive treatment with AMG9810 completely prevents chronic reflux-induced esophagitis

in rats suggesting that this is primarily due to activation of TRPV1 receptors (Yue et al., 2008). Similar result has been reported in mice (Fujino et al., 2005). In TRPV1 mutant mice reflux promoting surgery does produce esophagitis and in wild type mice treatment with capsaizine prevents esophagitis. We have shown in other study that in Sv40 T-antigen-immortalized human esophageal epithelial cells (HET-1A) TRPV1 is constitutively present. Exposure to acid induces over expression of TRPV1 receptors and secretion of pro-inflammatory cytokine IL-6 from these cells. Pretreatment with AMG 9810, not ASIC blocker benzamil hydrochloride, significantly blocks the IL-6 secretion suggesting that TRPV1 receptors are the main receptors involve in acid-induced esophagitis (Yue et al., 2008).

In conclusion, TRPV1 antagonist may play a key role in acid reflux-induced esophagitis and hypersensitivity. The use of TRPV1 antagonists could be an added and/or alternative to the traditional symptoms based treatment of chronic acid reflux and esophageal hypersensitivity.

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Figure 1.

Illustrates stimulus-response function (SRF) vagal afferent fiber to graded (10, 20, 30, 40 and 60mmHg) esophageal distension (ED). **A:** an example of intensity-dependant responses of ED-sensitive vagal afferent fiber from FL rat to ED. The fiber had ongoing spontaneous firing (15 imps/s) with the balloon placed in the distal esophagus. In each panel, top trace illustrates the nerve activity represented as frequency histogram (1s binwidth), middle trace is nerve action potentials and the bottom trace illustrates the esophageal distension (ED) pressures. The example does not illustrate the response of the fiber to 30mmHg distension. **B:** shows mean SRFs of fibers from naive control and FL rats. All fibers exhibited increasing firing to graded distension. However, responses of fibers from FL rats were not significantly higher than naive rats. **C:** summary data of SRFs in naive rats before and after injection of TRPV1 antagonists JYL1421 (10 mol/kg, iv). The drug did not influence responses of these fibers to distension. **D:** like JYL1421, AMG 9810 did not influence responses of ED-sensitive fibers to distension.

Figure 2.

Effect of CAP on ED-sensitive afferent fiber from naïve control rat before and after the application of TRPV1 selective antagonist JYL 1421. In each panel, the top trace represents the frequency histogram (1s binwidth), and the lower trace is the nerve action potentials. Arrows indicate the time of CAP injection. **A:** shows dose-dependent effect of CAP. CAP was injected at 40 minutes interval to avoid desensitization of response. **B:** shows the significant inhibition of response following JYL 1421 (10 μmol/kg, i.v.) injection. JYL 1421 was injected two minutes before the first dose of CAP.

Figure 3.

Effect of CAP on ED-sensitive afferent fiber from naïve control rat before and after the application of TRPV1 selective antagonist AMG9810. In each panel, the top trace represents the frequency histogram (1s binwidth), and the lower trace is the nerve action potentials. Arrows indicate the time of CAP injection. **A:** shows dose-dependent effect of CAP. CAP was injected at 40 minutes interval to avoid desensitization of response. **B:** shows the significant inhibition of response following AMG9810 (10 $\mu\text{mol/kg}$, i.v.) injection. AMG9810 was injected two minutes before the first dose of CAP.

Figure 4.

Summary data of dose-response functions of CAP before and after the application of TRPV1 antagonist in naïve control and fundus ligated (FL) rats. In FL rats, CAP produced significantly (# $p < 0.05$ vs. CAP response naïve control) greater response at lower doses (0.03 and 0.1 μmol/kg, iv) of CAP. JYL1421 (10 μmol/kg, iv) significantly attenuated responses of these fibers to CAP in both naïve (A) and FL (B) rats. Similarly, AMG 9810 (10 μmol/kg, iv) significantly blocked the CAP-induced excitation of vagal afferent fibers from naïve (C) and FL (D) rats. (* $p < 0.05$ vs pre-antagonist).

Figure 5.

Responses of two fibers from naïve control (**A**) and fundus ligated (FL, **B**) rats to infusion of acid (0.1ml of 0.1N HCl) before and two minutes after JYL1421 (10 μ mol/kg, i.v.) injection. In each panel, the top trace is the frequency histogram (1s binwidth) and the lower trace is the nerve action potentials. Arrows indicate acid infusion. The time interval between the acid infusion was 40 minutes. **C**: the summary data shows that JYL1421 does not attenuate responses of vagal afferent fibers from naïve and FL rats to acid (n=5/group).

Figure 6.

Effects of i.v. capsaicin (CAP) and intra-esophageal acid on ED-sensitive afferent fiber from fundus-ligated (FL) rat. In each panel, the top trace represents the frequency histogram (1s binwidth) and the lower trace is the nerve action potentials. **A** and **B**: show dose-dependent response of CAP (0.1 and 0.3 $\mu\text{mol/kg}$). The inter-stimulus interval between two doses of CAP was 40 minutes. **C**: shows excitation of the fiber to intra-esophageal infusion of acid (0.1ml of 0.1N HCl). **D**: shows complete block of excitation following application of AMG 9810 (10 $\mu\text{mol/kg}$, iv). Arrows indicate the time of acid infusion.

Figure 7.

A: Response of a vagal afferent fiber from fundus ligated (FL) rat to intra-esophageal acid (0.1N HCl, 0.1ml) before and after AMG 9810 (10µmol/kg, iv) injection. In each panel, the top trace represents the frequency histogram (1s binwidth), and the lower trace is the nerve action potentials. Arrows indicate the time of acid infusion. Acid produced excitation of the fiber that persisted 4–5 minutes. Following the injection of AMG 9810 (10µmol/kg, iv) the effect of intra-esophageal acid was significantly attenuated. The time interval between two acid applications was 40 minutes and AMG 9810 was injected two minutes before the second acid infusion. **B:** summary data of responses of four afferent fibers to acid before and after AMG 9810 injection. (* $p < 0.05$ vs pre-AMG9810).