Adenosine-induced activation of esophageal nociceptors

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Ru F, Surdenikova L, Brozmanova M, Kollarik M. Adenosine-induced activation of esophageal nociceptors. Am J Physiol Gastrointest Liver Physiol 300: G485–G493, 2011. First published December 9, 2010; doi:10.1152/ajpgi.00361.2010.—Clinical studies implicated adenosine acting on esophageal nociceptive pathways in the pathogenesis of noncardiac chest pain originating from the esophagus. However, the effect of adenosine on esophageal afferent nerve subtypes is incompletely understood. We addressed the hypothesis that adenosine selectively activates esophageal nociceptors. Whole cell perforated patch-clamp recordings and single-cell RT-PCR analysis were performed on the primary afferent neurons retrogradely labeled from the esophagus in the guinea pig. Extracellular recordings were made from the isolated innervated esophagus. In patch-clamp studies, adenosine evoked activation (inward current) in a majority of putative nociceptive (capsaicin-sensitive) vagal nodose, vagal jugular, and spinal dorsal root ganglia (DRG) neurons innervating the esophagus. Single-cell RT-PCR analysis indicated that the majority of the putative nociceptive (transient receptor potential V1-positive) neurons innervating the esophagus express the adenosine receptors. The neural crest-derived (spinal DRG and vagal jugular) esophageal nociceptors expressed predominantly the adenosine A1 receptor while the placodes-derived vagal nodose nociceptors expressed the adenosine A1 and/or A2A receptors. Consistent with the studies in the cell bodies, adenosine evoked activation (overt action potential discharge) in esophageal nociceptive nerve terminals. Furthermore, the neural crest-derived jugular nociceptors were activated by the selective A1 receptor agonist CCPA, and the placodes-derived nodose nociceptors were activated by CCPA and/or the selective adenosine A2A receptor CGS-21680. In contrast to esophageal nociceptors, adenosine failed to stimulate the vagal esophageal low-threshold (tension) mechanosensors. We conclude thatler adenosine selectively activates esophageal nociceptors. Our data indicate that the esophageal neural crest-derived nociceptors can be activated via the adenosine A1 receptor while the placodes-derived esophageal nociceptors can be activated via A1 and/or A2A receptors. Direct activation of esophageal nociceptors via adenosine receptors may contribute to the symptoms in esophageal diseases.

adenosine; nociception; esophagus

CLINICAL STUDIES HAVE PROVIDED evidence that adenosine stimulates pain pathways from the esophagus and contributes to functional chest pain originating from the esophagus (reviewed in Refs. 1 and 8). Intravenous adenosine induced esophageal hyperalgesia (33) similar to that observed in many patients with functional chest pain (26). Specifically, adenosine lowered the mechanical threshold for pain evoked by esophageal distention, indicating that adenosine influences esophageal nociceptive pathways (33). In high doses, intravenous adenosine induced chest discomfort and pain (36, 39) that was reduced by the methylxanthine nonselective adenosine receptor antagonist theophylline (36). Additional evidence for the role of adenosine in functional chest pain comes from studies using another methylxanthine nonselective adenosine receptor antagonist, theophylline (30). In patients with functional chest pain of presumed esophageal origin (Rome II), theophylline reduced esophageal mechanical hyperalgesia and reduced the frequency and intensity of pain (31, 32).

These observations are consistent with the notion that adenosine increases activity in the nociceptive pathways from the esophagus. Although the site of action of adenosine cannot be determined from these clinical studies, one explanation is that adenosine acts on the nerve terminals of afferent pain-mediating nerves in the esophagus. Thus far, the information on the effects of adenosine on the esophageal afferent nerves has been lacking (27). Accordingly, in the present study, we have focused on the activation of esophageal afferent nerves by adenosine.

The esophagus receives complex extrinsic sensory innervation supplied by vagal afferent neurons located in the vagal nodose and jugular ganglia and spinal afferent neurons located in the spinal dorsal root ganglia (DRG). Developmentally, vagal nodose ganglia are derived from embryonic placodes while vagal jugular and spinal DRG ganglia are derived from neural crest (2). In the guinea pig, the afferent nerves with nociceptive properties characterized by discriminative responses to mechanical and chemical stimuli are found in both vagal and spinal afferent pathways innervating the main body of the esophagus (reviewed in Ref. 22). Neural crest-derived spinal DRG and vagal jugular neurons project into the esophagus exclusively nociceptive nerve fibers, most of which are capsaicin-sensitive C fibers. In contrast, placodes-derived vagal nodose neurons project both capsaicin-sensitive C fiber nociceptors and capsaicin-insensitive A fiber mechanosensors. Our previous studies demonstrated that the placodes- and neural crest-derived nociceptors differ in activation profile and neurotransmitter chemistry (23, 43, 44).

In both vagal and spinal systems, we addressed the hypothesis that adenosine stimulates esophageal nociceptors. We found that adenosine activates both vagal and spinal esophageal nociceptors but not vagal low-threshold mechanosensors. Moreover, we found that the adenosine receptor profiles differ between the neural crest-derived (spinal DRG and vagal jugular) and placodes-derived (vagal nodose) nociceptors.

MATERIALS AND METHODS

All experiments described in this study were approved by the Johns Hopkins Animal Use and Care Committee and Jessenius Medical School Ethics Committee.

Retrograde labeling of the afferent neurons projecting into the esophagus was described previously (23, 43, 44). In a brief ketamine (50 mg/kg) and xylazine (2.5 mg/kg) anesthesia, the cervical esophagus was surgically exposed, and the retrograde tracer DiI (0.1% in

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A mixture of 10% DMSO in sterile saline was injected into the esophageal wall (1–2 sites up to the total volume of 5–10 µl). The esophageal surface was washed with a swab following the injection. The vagal nodose, vagal jugular, and spinal DRG (C8–T5) ganglia were harvested 10–15 days after esophageal DiI injections, enzymatically dissociated, and plated on cover slips. Dil labeling was identified by a fluorescent microscope. The neurons were used within 6 h for picking single neurons (RT-PCR analysis) and within 24 h for electrophysiology (patch clamp).

Single-cell RT-PCR was described previously (23, 25). First-strand cDNA was synthesized from single neurons by using the Super-Script III CellsDirect cDNA Synthesis System (Invitrogen) according to the manufacturer’s recommendations.

**Cell Picking**

Cover slips with dissociated neurons were perfused with Locke’s solution or PBS, and Dil-labeled neurons were individually harvested into a glass pipette (tip 50–150 µm) pulled with a micropipette puller (P-87, Sutter) by applying negative pressure. The pipette tip containing the cell was broken in a PCR tube containing resuspension buffer (1 µl; CellsDirect system) and RNase inhibitor (1 µl, RNaseOUT, 2 U/µl; Invitrogen), immediately frozen, and stored at −20°C. Only the neurons free of debris or attached cells were collected. From one cover slip, one to five cells were collected. A sample of the bath solution was collected in some cover slips for no-template experiments (bath control).

**RT-PCR**

Samples were defrosted, lysed (10 min, 75°C), and treated with DNase I. Next, poly(dT) and random hexamer primers (Roche Applied Bioscience) were added. The samples were reverse transcribed by adding SuperscriptIII RT for cDNA synthesis. In some experiments, a portion of the volume (50–75%) was reverse transcribed with RT, whereas RT was omitted in the remaining sample used as RNA control. Three microliters of each sample (cDNA, RNA control, or bath control) were used for PCR amplification by the HotStar Taq Polymerase Kit (Qiagen) according to the manufacturer’s recommendations in a final volume of 20 µl. After an initial activation step at 95°C for 15 min, cDNAs were amplified with custom-synthesized primers (see below, Invitrogen) by 50 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min followed by a final extension at 72°C for 10 min. Products were visualized in ethidium bromide-stained 1.5% agarose gels with 50- or 100-bp DNA ladder. Intron-spanning primers were designed by using Primer3 (34) based on the guinea pig sequences (forward primer, reverse primer, predicted product size, GenBank/EMBL/DDJB accession no. or CavPor3 scaffold): transient receptor potential (TRP) V1 (CAGAGGACCATCACCCTT, GGGGAGGGGAAAGTTC, 284 bp, AY729017), A1 (CATTGGGCCACACCCACTACT, 152 bp, scaffold 61), and A3a (CATCCTCCCTGCTATCACC, GCTGCTTCCTATGTTTC, 467 bp, D63674.1), A3b (CCTCCCTCCTGGCTGCTG, GGGACAGCATGACCCCTCT, 152 bp, scaffold 61), and A3 (TTCACCCATGCCTCAGCT, GAGGTGTTTCCTACCTCC, 188 bp, scaffold 2.320.1). The sequences of guinea pig adenosine receptors were deduced from CavPor3 assembly of the guinea pig genome (Feb. 2008 draft, UCSC Genome Bioinformatics Group) by using human, rat, and/or mouse adenosine receptor sequences and compared with database sequences if available. The identity of products amplified from single neurons was confirmed by sequencing in randomly selected samples (2–4) for each target. Figure 2 was prepared from multiple original gel images by using PowerPoint and Preview. Individual bands indicate only the presence or absence of a product.

Extracellular recordings from vagal neurons were described previously (43, 44). Extracellular recordings were made from vagal neurons with mechanosensitive nerve terminals in the esophagus in an isolated, perfused, vagally innervated guinea pig esophagus preparation. The esophagus and trachea were dissected with preserved bilateral extrinsic vagal innervation (including jugular and nodose ganglia). The tissue was pinned in a small Sylgard-lined Perspex chamber filled with indomethacin (3 µM) containing Krebs solution (in mM: 118 NaCl, 5.4 KCl, 1.2 MgSO4, 1.9 CaCl2, 25 NaHCO3, and 11 dextrose, gassed with 95% O2–5% CO2, pH = 7.4, 35°C). The esophagus with attached trachea and the vagus were pinned in the tissue compartment, and the rostral aspect of the vagus nerves, including the nodose and jugular ganglia, were pinned in the recording compartment. The two compartments were separately superfused with Krebs solution (pH = 7.4, 35°C, 4–6 ml/min). Polyethylene tubing was inserted 3–5 mm in the cranial and caudal esophagus and secured for perfusion. The pressure in the fluid (Krebs)-filled esophagus was measured with a differential pressure transducer connected in series to the esophagus and recorded simultaneously with neural activity by the chart recorder (TA240S; Gould, Valley View, OH). Isobaric esophageal distension for 20 s with an intraluminal pressure of 10–30 or 60–100 mmHg (generated by a gravity-driven pressure system) separated by 3 min was used to determine the distension pressure-nerve activity relationship of an esophageal afferent fiber. An aluminum-silicate glass microelectrode was filled with 3 M sodium chloride (electrode resistance 2 MΩ). The electrode was placed in an electrode holder connected directly to the head stage (A-M Systems). A return electrode of silver-silver chloride wire and earthed silver-silver chloride pellet was placed in the perfusion fluid of the recording compartment. The recorded signal was amplified (M1800; A-M Systems) and filtered (low cutoff, 0.3 kHz; high cutoff, 1 kHz), and the resultant activity was displayed on an oscilloscope (TDS340; Tektronix) and the chart recorder. The data were stored and analyzed on an Apple computer using the software TheNerveOff (sampling frequency 33 kHz; PHOCIS, Baltimore, MD).

**Recording from the Spinal DRG Nociceptors**

The esophagus with adjacent tissue (at the level of spinal ganglia approximately C8–T3) that included a portion of the left sympathetic trunk, and left spinal T1–T5 DRG ganglia were carefully dissected. Caution was made to preserve the tissue adjacent to the esophagus containing spinal afferent nerve pathways. The esophagus was secured dorsal side up in the tissue chamber. The DRG (T2 or T3) ganglion with a short (2–3 mm) portion of its spinal nerve was pulled through a small hole into a separately perfused Sylgard-lined recording chamber and pinned. The hole was then sealed with vaseline. The recording electrode was micromanipulated into the nodose or jugular ganglion, and a distension-sensitive unit was identified when esophageal distension (60–100 mmHg for 5 s) evoked action potential discharge. The outer surface of the esophagus was searched with a focal mechanical probe (Von Frey hair, 1 mN, filament diameter <0.5 mm). A mechanosensitive receptive field was located when the punctate stimulus evoked discharge of action potentials with waveform forms identical to the action potentials evoked by distension.
as the maximal 1-s bin action potential discharge. The response to the particular pharmacological/chemical stimulus was considered positive when the stimulus evoked action potential discharge with a peak frequency of at least 2 Hz (in the fibers with no baseline activity) or a peak frequency at least three times the frequency of baseline activity. Data are presented as mean ± SE maximum discharge in a 1-s bin (nociceptors) and 10- and 60-s bins (mechanosensors). Paired, unpaired t-test and one-way ANOVA were used as appropriate.

The possibility that adenosine causes mast cell activation in the guinea pig esophagus was addressed by using the histamine release assay described previously (41). The amount of histamine released from the esophageal tissue by adenosine (10 or 100 µM, 20 min, n = 4) was equal to the baseline release over the same period (<1% of the total tissue histamine content). For comparison, in an identically designed experiment, antigen caused histamine release on average 17% of the tissue histamine content in preparations from sensitized animals (41).

Whole cell patch-clamp recording was described previously (23, 43). To preserve intracellular signaling pathways, a gramicidin-perforated whole cell patch-clamp technique was employed (23). The recordings were performed using a Multiclamp 700A amplifier and Axograph 4.9 software. The pipette (1.5–3 MΩ) was filled with a pipette solution composed of (in mM) 140 KCl, 1 CaCl2, 2 MgCl2, 11 EGTA, 10 HEPES, and 10 dextrose titrated to pH 7.3 with KOH (304 mosmol/l) containing 2–3.5 µg/ml gramicidin. Gramicidin was dissolved in DMSO (1 mg/ml) and mixed with the pipette solution just before each recording. After a gigasohm seal was formed, cell membrane potential was held at −60 mV. The inclusion criteria were the series resistance <30 MΩ and the membrane resistance >100 MΩ. In voltage-clamp mode, recordings were made after whole cell capacitance compensation. In current-clamp mode, the neurons were held at −65 mV. During the experiments, the cells were continuously superfused (6 ml/min) with Locke’s solution (35–37°C) composed of (in mM) 136 NaCl, 5.6 KCl, 1.2 MgCl2, 2.2 CaCl2, 1.2 NaH2PO4, 14.3 NaHCO3, and 10 dextrose gassed with 95% O2-5% CO2 (pH 7.3–7.4). Drugs were delivered in superfusing solution: adenosine (Sigma) was dissolved to 10 mM in water at the day of use. α,β-me-ATP (Sigma) was dissolved in water (stock solution 10 mM), and capsaicin (Sigma) was dissolved in ethanol (stock solution 10 mM). CCPA (Tocris) and CGS-21680 (Tocris) were dissolved in DMSO (stock solutions 10 mM). Stock solutions were stored at −20°C. All drugs were further diluted in Locke’s of Krebs buffer to indicated final concentrations shortly before use.

Statistics

The frequency of expression of adenosine receptor subtypes was compared by the Chi-squared test. The sensitizing effect of the selective adenosine receptor agonist was evaluated by paired t-test.

RESULTS

Single Neuron Electrophysiology

In the guinea pig, vagal nodose ganglion neurons project capsaicin-insensitive tension A fiber mechanoreceptors and capsaicin-sensitive C fiber nociceptors (43, 44). Vagal jugular ganglion neurons and spinal DRG neurons project almost exclusively capsaicin-sensitive nociceptors (43). Whole cell gramicidin-perforated patch-clamp recordings were made from the sensory neurons retrogradely labeled from the esophagus. Adenosine induced an inward current in the majority of nodose (6/8), jugular (12/14), and DRG (13/17) nociceptive (capsaicin-sensitive) neurons (Fig. 1). The inward current averaged between 10 and 20 pA/pF (Fig. 1B). For comparison, capsaicin (1 µM) that we found in our system, the most effective pharmacological stimulus of esophageal nociceptive neurons, evoked inward currents with peak current density 77 ± 22 pA/pF, 71 ± 12 pA/pF, and 80 ± 9 pA in the studied nodose, jugular, and DRG neurons, respectively. In contrast to capsaicin-sensitive neurons, adenosine failed to stimulate nodose capsaicin-insensitive neurons (n = 21, 17 of these neurons responded to control stimulus α,β-me-ATP). These data indi-

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cate that cell bodies of the three nociceptive subtypes innervating the esophagus are directly stimulated by adenosine. In contrast, the putative cell bodies of vagal low-threshold mechanosensors in the esophagus are unresponsive to adenosine.

**Single-Neuron RT-PCR Analysis**

Because of their responsiveness to adenosine, we focused our RT-PCR analysis on the putative nociceptive capsaicin-sensitive (TRPV1-positive) neurons. The expression of mRNA for all four known adenosine receptors (A1, A2A, A2B, and A3) (15, 30) was evaluated in the TRPV1-positive neurons retrogradely labeled from the esophagus in a separate set of experiments (Fig. 2).

**Nodose neurons.** The adenosine receptors A1 and A2A were the major subtypes expressed in TRPV1-positive neurons. mRNA for A1 and/or A2A receptors was detected in the majority (12/15) of the esophagus-specific TRPV1-positive nodose neurons. Of these 12 neurons, 7 coexpressed A1 and A2A, whereas 2 neurons expressed A1 only and 3 neurons expressed A2A only. None of the esophagus-specific TRPV1-positive nodose neurons expressed A2B receptor (n = 15) or A3 receptor (n = 10, 4 of tested neurons are shown in Fig. 1). In control experiments, A2B and A3 were readily detected in the samples from the whole sensory ganglia (DRG) and in some individual DRG neurons (Fig. 2).

**Jugular and DRG neurons.** The majority (11/14) of the esophagus-specific TRPV1-positive jugular neurons was positive for the adenosine A1 receptor. The adenosine A2A and A2B receptors were detected only in small proportions (3/14 and 3/14, respectively) of these neurons, and A3 receptor was not detected. Similar to jugular nociceptors, the esophagus-specific TRPV1-positive DRG neurons innervating the esophagus expressed A1 receptor (9/17) but rarely A2A (1/17) and A2B (3/17) receptors and did not express A3 receptor.

These results indicate that the majority of the nodose nociceptive neurons express the adenosine A1 and/or A2A receptors. In contrast, the majority of the jugular and DRG neurons express A1 receptor that in a small proportion of neurons combines with A2A or A2B receptors. The adenosine A2A receptor was significantly (P < 0.01, Chi test) more frequently expressed in nodose nociceptive neurons than in jugular and DRG nociceptive neurons. The frequency of expression of A1 receptor was not significantly different between nodose nociceptive neurons and jugular and DRG nociceptive neurons.

**Action Potential Discharge from Nerve Terminals in the Esophagus**

Consistent with the studies on the neuronal cell bodies, adenosine activated vagal nociceptive subtypes in the esophagus (Fig. 3). Adenosine (100 μM, 10–15 min) induced action potential discharge in 6 of 9 capsaicin-sensitive nodose nociceptive fibers (peak frequency 4.0 ± 0.6 Hz) and in 7 of 12 capsaicin-sensitive jugular nociceptive fibers (peak frequency 4.6 ± 0.5 Hz). The pattern of action potential discharge was variable; either a relatively regular discharge (illustrated in a nodose nociceptive fiber; Fig. 3A) or an irregular discharge with occasional bursts of action potentials (illustrated in a jugular nociceptive fiber; Fig. 3A) was observed. There was no...
obvious difference in discharge pattern between nodose and jugular nociceptive fibers. The onset of discharge was variable (0.5–5 min, the fibers with relatively rapid onset are shown in Fig. 3A). The variable delay in the onset of the action potential discharge is a consistent observation in our preparation (42–44) attributable to the time required for equilibration and diffusion of the agonist to the nerve terminals in the esophageal wall (44).

The magnitude of activation was compared with that evoked by esophageal distention to 60 mmHg (Fig. 3) that is considered the threshold noxious level for evoking pain in humans and nocifensive responses in animals. Nodose nociceptors respond to step esophageal distention (for 20 s) with action potential discharge averaging 38 action potentials (range 3–113). As expected from our previous studies, all esophagus-adapting sustained discharge, the peak frequency of adenosine-induced discharge was comparable to that induced by the threshold noxious esophageal distention (60 mmHg, nodose, n = 6, jugular, n = 7) relative to that induced by the threshold noxious esophageal distention (60 mmHg, nodose, n = 35, jugular, n = 13). In nodose nociceptors, the peak discharge reflects the dynamic phase of the mechanical response (see text for details). *P < 0.05 compared with the mechanical response. The average baseline discharge frequency of nodose and jugular nociceptors was 0.1 ± 0.05 and 0.07 ± 0.03 Hz, respectively.

Adenosine receptor agonists also had a moderate sensitizing effect on mechanical response of esophageal nociceptors. We have previously demonstrated that the response of nodose and jugular nociceptors to esophageal distention is very reproducible (44). The response to esophageal distention with intraluminal pressure of 60 mmHg was tested in the absence and in the presence of agonists in paired experiments. The adenosine A1 receptor agonist CCPA (0.1–0.3 μM, peak discharge frequency 3–9 Hz), and 5/9 responded to the selective adenosine A2A receptor agonist CGS-21680 (0.1 μM, peak frequency averaging 3 Hz). Four of the responsive fibers were tested for both agonists: 2 responded to both CCPA and CGS-21680, and 2 responded to CGS-21680 only. Four of 8 jugular capsaicin-sensitive nociceptive fibers responded to CCPA (0.3 μM, peak frequency 3–4 Hz), but these fibers rarely responded to the A2A agonist CGS-21680 (1 of 6 responded). Figure 4 illustrates a nodose nociceptive fiber responsive to both CCPA and CGS-21680 and a jugular nociceptive fiber responsive to CCPA but not CGS-21680. Nodose (n = 9) and jugular (n = 8) nociceptive fibers evaluated in this set of experiments displayed only trivial baseline discharge averaging 0.06 ± 0.02 and 0.03 ± 0.02 Hz, respectively.

Adenosine receptor agonists were chosen based on the previous pharmacological analysis of adenosine receptors in the guinea pig vagal afferent nerves (see discussion in Ref. 9 for details). Three of 6 nodose capsaicin-sensitive nociceptive fibers responded to the selective adenosine A1 receptor agonist CCPA (0.1–0.3 μM, peak discharge frequency 3–9 Hz), and 5/9 responded to the selective adenosine A2A receptor agonist CGS-21680 (0.1 μM, peak frequency averaging 3 Hz). Four of the responsive fibers were tested for both agonists: 2 responded to both CCPA and CGS-21680, and 2 responded to CGS-21680 only. Four of 8 jugular capsaicin-sensitive nociceptive fibers responded to CCPA (0.3 μM, peak frequency 3–4 Hz), but these fibers rarely responded to the A2A agonist CGS-21680 (1 of 6 responded). Figure 4 illustrates a nodose nociceptive fiber responsive to both CCPA and CGS-21680 and a jugular nociceptive fiber responsive to CCPA but not CGS-21680. Nodose (n = 9) and jugular (n = 8) nociceptive fibers evaluated in this set of experiments displayed only trivial baseline discharge averaging 0.06 ± 0.02 and 0.03 ± 0.02 Hz, respectively.

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Adenosine also induced overt action potential discharge in the cell bodies and the nerve terminals of the spinal DRG nociceptors innervating the esophagus (Fig. 6). In the whole cell gramicidin-perforated current-clamp recordings (~65 mV), adenosine (10 μM, 60 s) induced depolarization averaging 12 ± 2 mV in five of six DRG neurons retrogradely labeled from the esophagus. In four of these five DRG neurons depolarized by adenosine, this depolarization resulted in overt action potential discharge averaging 38 action potentials (range 3–113). As expected from our previous studies, all esophagus-
labeled DRG neurons (n = 5) tested for the response to capsaicin (1 μM) responded with depolarization 58 ± 1 mV leading to an average 37 action potentials (range 20–58). In the preparation of spinally (T2–T3)-innervated isolated esophagus (see MATERIALS AND METHODS for details), adenosine (100 μM, 20 min) induced over action potential discharge in 4 of 10 spinal DRG fibers with the mechanosensitive nerve terminals in the esophagus with the peak frequency averaging 3 Hz. As expected, capsaicin (1 μM, 15 min) activated all evaluated DRG fibers (peak frequency of action potential discharge was 10 ± 2 Hz, n = 10). Spinal DRG fibers had virtually no baseline activity (average baseline discharge ~0.01 Hz).

In contrast to its excitatory effect on esophageal nociceptors, adenosine failed to effectively stimulate the esophageal vagal low-threshold (tension) mechanosensors (Fig. 7). Similar to other species, the majority of the guinea pig esophageal mechanosensors display regular baseline discharge attributed to mechanical forces in the tissue under resting conditions (Fig. 7A, inset). Adenosine failed to appreciably alter this baseline discharge. However, the mechanosensors were effectively stimulated by low-level control stimuli, the P2X receptor agonist α,β-me-ATP, and the esophageal distention with an innocuous pressure (10 mmHg) (Fig. 5). These results are consistent with the lack of effect of adenosine on the putative neuronal cell bodies of the tension mechanosensors (nodule esophagus-...
specific capsaicin-insensitive neurons; Fig. 1, A and C). Adenosine did not significantly alter the response of tension mechanosensors to esophageal distention (data not shown).

**DISCUSSION**

We have found that adenosine selectively activates esophageal nociceptors. Our data showing the expression of adenosine receptors (mRNA) and the response to adenosine in isolated esophagus-specific nociceptive neurons support the conclusion that this activation is due to a direct effect of adenosine on the nociceptors. These results are consistent with the notion that the probable sites of action of adenosine in pathogenesis of chest pain are the nociceptive nerve terminals in the esophagus. Our data also indicate that the esophageal neural crest-derived (spinal DRG and vagal jugular) nociceptors are activated mainly via the adenosine A1 receptor, whereas the placodes-derived (vagal nodose) nociceptors are activated via both adenosine A1 and A2A receptors. These observations further highlight the differences between the neural crest- and placodes-derived nociceptive subtypes in the esophagus (22).

We found that, compared with capsaicin, adenosine was a relatively modest activator of esophageal nociceptive nerve terminals. Yet, the maximum magnitude of adenosine-induced activation was comparable to that induced by esophageal distention to the level considered threshold for esophageal pain in humans and nociceptive responses in animal models (60 mmHg). A moderate sensitizing effect of the adenosine A1 receptor on the nociception caused by mechanical stimuli and a higher concentration of adenosine was required to induce transient chest pain (36, 39).

The effect of adenosine on primary afferent nerves in the esophagus was selective for those nerves with a nociceptive phenotype. Adenosine had no effect on the esophageal low-threshold mechanosensors. This lack of effect was unlikely due to limitations in drug delivery to the terminals because the purinergic P2X receptor agonist α,β-me-ATP strongly activated these nerves. Moreover, the nerve cell bodies of mechanosensors (esophagus-specific capsaicin-insensitive nodose neurons) failed to respond to direct application of adenosine in our patch-clamp analysis.

Vagal jugular ganglia and spinal DRG ganglia are derived from the embryonic neural crest while vagal nodose ganglia are derived from the embryonic placodes (2). Available data suggest that the neural crest- and placodes-derived nociceptors mediate different aspects of nociception (reviewed in Ref. 22). We also found that the embryonic origin dictates certain sensory properties of nociceptors in the guinea pig esophagus (23, 43, 44). Thus the vagal jugular nociceptors are similar to DRG nociceptors but distinct from the vagal nodose nociceptors. With respect to activation profile, the neural crest- and placodes-derived nociceptors differ in response to purinergic agonists (via P2X2/3 receptor) and serotonin (via the 5-HT3 receptor). These stimuli overtly activate the placodes-derived (vagal nodose) nociceptors, but not the neural crest-derived (vagal jugular and DRG) nociceptors. Our present study indicates that the difference in activation mechanisms between the nociceptive subtypes also includes the adenosine receptor types mediating the response to adenosine. Although both nociceptive subtypes are activated by adenosine, our data are consistent with the conclusion that the neural crest-derived (vagal jugular and DRG) nociceptors are activated via the adenosine A1 receptors, whereas placodes-derived (vagal nodose) nociceptors can be activated via the adenosine A1 and/or A2A receptors.

The conclusion that the placodes-derived (nodose) nociceptors are activated via adenosine A1 and/or A2A receptors and neural crest-derived (vaginal and DRG) nociceptors via primarily the adenosine A1 receptors is supported by a combination of data from single-cell RT-PCR studies (Fig. 2) and extracellular recording studies with the selective agonists of the adenosine A1 and A2A receptors (Fig. 4). The assertion that at the concentrations employed the adenosine A1 receptor agonist CCPA (0.1–0.3 μM) and the adenosine A2A receptor agonist...
CGS-21680 (0.1–0.3 μM) are selective for the adenosine A₁ and A₂A receptors, respectively, is based on the previous pharmacological analysis using a similar experimental design in which guinea pig vagal afferent fibers in the respiratory tract were evaluated (9). The adenosine A₂B receptor was detected only in a minority (<20%) of the neural crest-derived nociceptive neurons, and the adenosine A₃ receptor was absent, indicating that A₂B and A₃ receptors play only a minor (if any) role in direct modulation of the esophageal nociceptors. Our finding that the nodose nociceptors in the esophagus are activated via the adenosine A₁ and/or A₂A receptors is similar to the observation made in the guinea pig lungs (9). In contrast to our finding in the esophagus, adenosine appears to be an ineffective activator of vagal jugular C fibers in the respiratory system. This potential difference in the jugular nociceptive phenotype between the respiratory and gastrointestinal system may indicate that certain properties of vagal nociceptors are organ- or tissue-specific and warrants further investigation in the future. It has been previously reported that visceral spinal DRG afferent nerves are activated by adenosine (21). Our finding that the spinal DRG (i.e., neural crest-derived) afferent neurons are activated via the adenosine A₁ receptor is consistent with the previous reports in rat jejunum (6).

The conclusion that the stimulation of adenosine receptors directly on the nerve terminals leads to activation of esophageal nociceptors is supported by the expression of the adenosine receptors and direct excitatory effects of adenosine in their neurons. Although unlikely, in a complex tissue such as esophagus, the contribution of a secondary activation (due to presynaptic neurons) to the nociceptive response may indicate that certain properties of vagal nociceptors are organ- or tissue-specific and warrants further investigation in the future. It has been previously reported that visceral spinal DRG afferent nerves are activated by adenosine (21). Our finding that the spinal DRG (i.e., neural crest-derived) afferent neurons are activated via the adenosine A₁ receptor is consistent with the previous reports in rat jejunum (6). The conclusion that the stimulation of adenosine receptors directly on the nerve terminals leads to activation of esophageal nociceptors is supported by the expression of the adenosine receptors and direct excitatory effects of adenosine in their neurons. Although unlikely, in a complex tissue such as esophagus, the contribution of a secondary activation (due to presynaptic neurons) to the nociceptive response may indicate that certain properties of vagal nociceptors are organ- or tissue-specific and warrants further investigation in the future. It has been previously reported that visceral spinal DRG afferent nerves are activated by adenosine (21). Our finding that the spinal DRG (i.e., neural crest-derived) afferent neurons are activated via the adenosine A₁ receptor is consistent with the previous reports in rat jejunum (6).

Pain from visceral organs is thought to be mediated by spinal DRG nociceptors, and these afferent nerves are most likely the main pathway for chest pain from the esophagus. The consequences of the activation of esophageal vagal nociceptors have not been defined yet (discussed in Ref. 22). Vagal afferent inputs have been implicated in the modulation of pain perception, and thus any activity in vagal esophageal nociceptors may modulate the pain. It is also probable that, similar to the neighboring respiratory system in which vagal nociceptors (bronchopulmonary C fibers) modulate regulatory reflexes (10, 24), vagal esophageal nociceptors may modulate the reflex regulation of the esophagus. In this context, it is noteworthy that certain esophageal muscle function abnormalities noted in patients with functional chest pain (26, 33) and that the drug-antagonizing adenosine receptors have beneficial effects in these patients (31, 32). The source of adenosine in the esophagus is at present unknown. Metabolism of ATP is considered to be the most important source of extracellular adenosine in the body (35) since ATP can be released from the intracellular compartment under various types of stress (5, 7) and converted extracellularly to adenosine. Further clinical studies are needed to identify cellular source(s) of adenosine in patients with chest pain of esophageal origin.

The role of adenosine in nociception and pain is complex. In human studies, adenosine was reported to induce pain from somatic (4, 29, 37) and visceral (12, 18, 36) tissues, which has been in part attributed to the stimulation of the adenosine A₁ receptor on peripheral sensory nerves (18, 29). In contrast, stimulation of adenosine A₂B receptor in the spinal cord typically leads to inhibition of nociceptive signaling (reviewed in Refs. 14 and 17), and central antinociceptive effects of adenosine have been attributed to these mechanisms. The stimulation of the adenosine A₁ receptor in neurons affects various potassium, mixed cationic, or calcium currents through activation of G proteins coupled to ion channels, adenyl cyclase, or phospholipases (19). In peripheral nociceptors, the stimulation of the adenosine A₂B receptor has been reported in some instances to stimulate somatic nociceptors [such as nociceptors innervating the knee (13)] and visceral nociceptors [such as nociceptors innervating the jejunum (6)], whereas in other instances it may inhibit nociceptive responses [such as in the rat skin (20, 38)]. The effect of stimulation of the adenosine A₁ receptor in a specific type of nociceptor most likely depends on its coupling to particular effectors. The adenosine A₂A receptor has been reported to have pronociceptive effects in the peripheral somatosensory system (20, 38).

In the visceral sensory system, adenosine has been implicated in cardiac pain. Some clinical studies suggest that adenosine stimulates cardiac afferent nerves (12, 18) although this has not been consistently observed across the studies (11, 40). Available experimental data show that adenosine is not an effective activator of cardiac nociceptive nerves (3, 28) (reviewed in Ref. 16).

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DISCLOSURES

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