Localization and functional effects of adenosine A1 receptors on cardiac vagal afferents in adult rats

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Middlekauff, Holly R., Scott A. Rivkees, Helen E. Raybould, Melo Bitticaca, Joshua I. Goldhaber, and James N. Weiss. Localization and functional effects of adenosine A1 receptors on cardiac vagal afferents in adult rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H441–H447, 1998.—There is evidence to suggest that during ischemia adenosine acts on cardiac vagal afferent neurons to activate systemic reflexes and to modulate cardiac nociception. The purpose of this study was to determine whether adenosine receptors are present and have direct cellular electrophysiological actions on cardiac vagal afferent neurons. In radioreceptor assays of nodose ganglion tissue from rats, binding was detectable for A1 (39.6 ± 1.2 fmol/mg protein) but not A2a adenosine receptors. These findings were confirmed using the complementary approach of receptor-labeling autoradiography. Using in situ hybridization, we saw specific labeling over ~50% of neurons in the nodose ganglia, but not over nonneuronal cells. In colabeling studies, cardiac vagal afferent neurons were identified by retroneuronal labeling with fluororuby. Of cardiac vagal afferents approximately one-half were strongly positive for A1 adenosine receptors (immunocytochemistry). In patch-clamping experiments, adenosine inhibited peak inward calcium current in 7 of 11 neurons by 48 ± 13%. In conclusion, adenosine A1 receptors reside on a subset of vagal afferent neurons, including cardiac vagal afferents, and have electrophysiological effects that modulate nerve excitability in cultured nodose ganglion neurons.

adeno sine, an endogenous nucleoside, is generated from the breakdown of ATP during myocardial ischemia. Adenosine has been called the "retaliatory metabolite" because many of its actions serve to restore the energy balance of the system to normal (15). Adenosine increases energy supply by acting on blood vessels to produce vasodilatation, and adenosine decreases energy demand by its negative chronotropic and dromotropic effects on the cardiac conduction system, negative inotropic effects on ventricular myocytes, and inhibitory effects on norepinephrine release from efferent sympathetic nerve terminals (2, 3, 20). In addition, there is evidence to suggest that adenosine acts on cardiac vagal afferent neurons to activate systemic reflexes (19). Finally, because a role for vagal afferents in mediating cardiac pain has also been described (14), adenosine may act on cardiac vagal afferents to modulate cardiac nociception as well (6). It is unknown whether adenosine interacts directly with cardiac vagal afferents or works through intermediaries. The purpose of this study was to determine whether adenosine receptors are present and have cellular electrophysiological actions on cardiac vagal afferent neurons.

METHODS

Radioreceptor assays. Adult Sprague-Dawley rats (180–220 g) were anesthetized with pentobarbital sodium (100 mg/kg) and decapitated. Bilateral nodose ganglia were removed and placed immediately into iced Dulbecco's modified Eagle's medium (DMEM). Ganglia were minced and placed in dispersion solution, consisting of 5 ml DMEM, 0.5 mg/ml trypsin, 1 mg/ml collagenase, and 0.1 mg/ml deoxyribonuclease (DNase), and incubated at 35°C for 30 min in a shaker bath. Trypsin inhibitor (2.5 mg) was then added to the cell suspension in 5 ml DMEM. Cells were gently mechanically dissociated, placed in microfuge tubes, and frozen at −80°C.

To facilitate binding studies using small amounts of tissue, we used crude tissue preparations. Chilled (4°C) binding buffer that contained 5 U/ml of adenosine deaminase (ADA; Boehringer Mannheim, Indianapolis, IN) was then added (1 ml/tube), and specimens were sonicated using a Sonics and Materials sonicator (power = 40, 2 × 5 s; 4°C; Danbury, CT). Preparations were then incubated in a rolling incubator at 37°C for 45 min. One-hundred microliters of crude tissue preparation were then incubated with [3H]-labeled 8-cyano-1,3-dipropylxanthine ([3H]DPCPX; DuPont NEN; Wal tham, MA), a selective A1 adenosine receptor ligand, or with [3H]-labeled 2-(4-[2-carboxyethyl]phenylethylamino)-5′-N-ethyl carbamidoadenosine ([3H]CGS-21680; DuPont NEN), a selective A2a adenosine receptor ligand, in Millipore MultiScreen plates with Whatman GF/B glass fiber filter bottoms (Millipore; New Bedford, MA). To determine nonspecific binding, we included 10 µM unlabeled DPCPX or CGS-21680 in some incubations. Bound radioactivity was separated from free using a vacuum manifold. The amount of radioactivity present was determined by liquid scintillation counting. In each study total and nonspecific binding was tested in quadruplicate, and each specimen was tested at least three times. Specific binding was calculated as the difference between total and nonspecific binding. Protein concentrations were determined using bicinchoninic acid (Pierce; Rockford, IL).

Receptor-labeling autoradiography. Receptor-labeling autoradiography was performed as previously described (17). Adult Sprague-Dawley rats (180–220 g) were anesthetized with pentobarbital sodium and decapitated. Bilateral nodose ganglia were dissected and placed immediately into iced DMEM. Tissue underwent ultrarapid freezing on a Life Cell CF100 cryofixation unit and was stored in liquid nitrogen. Sections (10 µm) were then cut on a cryostat and thaw mounted on Superfrost/Plus slides (Fisher Scientific, Itaska, IL) and stored at −80°C. Tissue sections were thawed at room temperature and incubated in Dulbecco's phosphate-buffered saline (DPBS) with 10 mM MgCl2 (binding buffer) that contained 1% formaldehyde (3 min, 21°C). Slides were then placed in binding buffer containing ADA (2 U/ml; 30 min, 21°C). Sections were next incubated in binding buffer contain-
ing ADA (2 U/ml) and [\(^{3}H\)DPCPX or [\(^{3}H\)CGS-21680 (4 nM, 60 min, 21°C). Non-specific binding was assessed from incubations that also contained 10 \(\mu\)M DPCPX or CGS-21680. Sections were then washed in DPBS (2 \(\times\) 15 min at 0°C), air dried, and exposed to \(^{35}S\)-labeled Hyperfilm (Amersham; Rockford, IL) for 90–180 days. Films were developed using Kodak D-19 developer (4 min, 21°C).

**In situ hybridization.** The cDNA encoding the full-length rat \(\alpha_1\) adenosine receptor was generously provided by Steven M. Reppert at Massachusetts General Hospital (Boston, MA). This cDNA has been previously characterized in full (16). Tissue sections (10 \(\mu\)m) were obtained as described in Receptor-labeled autoradiography and were stored at \(-80°C. Uridine 5'-(\[^{35}S\]thiotriphosphate ([\(^{35}S\]UTP, ammonium hydroxide.

The intensity of labeling was graded as strongly positive, weakly positive, or absent by two investigators (Bitticaca and Middlekauff).

Tissue culture. Tissue culture was performed according to the method of Yoshimura et al. (22). Briefly, 2–3 wk after FR dye injections, rats were anesthetized with pentobarbital and decapitated. Nodose ganglia were removed and placed immediately into iced DMEM. Ganglia were minced, placed in dispersion solution consisting of 5 mL DMEM, 0.5 mg/ml trypsin, 1 mg/ml collagenase, and 0.1 mg/ml DNase, and incubated at 35°C for 30 min in a shaker bath. Trypsin inhibitor (2.5 mg) was then added to the cell suspension in 5 mL DMEM. Cells were gently mechanically dissociated and plated on matrigel-coated cover slips. Cells were used for electrophysiological studies within 2–24 h after isolation. The culture medium consisted of minimal essential media with 10% horse serum and 10% fetal bovine serum supplemented with 20 \(\mu\)l/ml of penicillin-streptomycin solution.

**Cellular electrophysiology.** Both FR-labeled and unlabeled neurons were used in patch-clamping experiments. Whole-cell voltage- and current-clamp recordings were performed at room temperature. Patch electrodes had a resistance of 2–3 M\(\Omega\). Membrane current and voltage were measured with an Axopatch 200 (Axon Instruments) patch-clamp amplifier controlled by a personal computer using a Labmaster analog-to-digital board driven by pCLAMP software (Axon Instruments). Isolated neurons were examined with a Nikon inverted microscope equipped with epifluorescence illumination using a xenon lamp (excitation 555 nm) with emission detected at 580 nm. The standard external solution contained (in mM) 136 (NaCl + NaOH), 5.4 KCl, 0.33 NaH\(_2\)PO\(_4\), 1.0 MgCl\(_2\), 10.0 N-2-hydroxymethylaminomethane buffer-N^\(-2\)-ethanesulfonic acid (HEPES), 10.0 glucose, and 1.8 CaCl\(_2\), pH 7.4. The internal solution was prepared (in mM) 140 (KCl + KOH), 5.0 ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1.0 CaCl\(_2\), 0.5 MgCl\(_2\), 10.0 HEPES, 5 ATP, 0.1 GTP, and 0.01 adenosine 3',5'-cyclic monophosphate, pH 7.2. For isolation of calcium currents (I\(_{\text{Ca}}\), Na and K in the external solution were replaced isotonically with Cs, 0.03 mM tetrodotoxin (TTX) was added, and CaCl\(_2\) was increased to 5 mM; in the internal solution, K was replaced with 30 mM tetraethylammonium (TEA) and 110 mM Cs. In current-clamp mode, action potentials were stimulated by current injection. In voltage-clamp experiments, calcium currents were elicited by depolarizing voltage steps from a holding potential of \(-80\) mV after blocking TTX-sensitive Na currents and K currents.

**Immunocytochemistry.** After a 2- to 3-wk survival time to allow retrograde axonal transport of FR to the neuronal cell bodies, rats were euthanized and tissue was processed for immunocytochemistry or cellular electrophysiology as described below.

**Immunocytochemistry.** After a 2- to 3-wk survival time to allow retrograde axonal transport of FR to the neuronal cell bodies, rats were euthanized with pentobarbital sodium perfused transcardially with 300 ml 0.1 phosphate-buffered saline (PBS), pH 7.4, and then 300 ml 4% paraformaldehyde in 0.1 M PBS, pH 7.4, at 4°C. Bilateral nodose ganglia were dissected and placed in 4% paraformaldehyde for 2 h and then transferred to 20% sucrose-PBS overnight at 4°C. Ganglia were embedded, and 10-\(\mu\)m sections were cut on a cryostat and thaw mounted on Superfrost/Plus slides. Sections were viewed with a Nikon microscope equipped with epifluorescence with a Mercury arc lamp, illuminating cells at 555 nm. Fluorescence was measured at 580 nm. FR-positive cells were prospectively defined as cells with bright granular label throughout the cytoplasm, as described previously (4).

Selective, polyclonal \(\alpha_1\) adenosine receptor antibody was prepared by Rivkees and colleagues (18) as previously described. Tissue sections were rinsed four times (for 3, 5, 7, and 7-min) with PBS containing 0.02% Tween (Sigma) and were incubated overnight with adenosine \(\alpha_1\) receptor antisera at room temperature. The primary antisera dilution was 1:1,000 in PBS-Tween and 1.5% goat serum (Vector). Sections were then rinsed and incubated with secondary goat anti-rabbit antisera (1:200 dilution in PBS-Tween with 1.5% goat serum) for 90 min at room temperature. Tissue was then rinsed and incubated with avidin-biotin complex (1:150 dilution) in PBS-Tween for 90 min. Sections were again rinsed with PBS-Tween and then incubated with 3,3'-diaminobenzidine (50 mM tris(hydroxymethyl)aminomethane buffer; Sigma) for 5–30 min. Sections were rinsed in 0.01 M PBS, air dried, and coverslipped. In control experiments, the secondary antibody was omitted. Slides were examined by light microscopy.

For prehybridization, sections were treated with HCl and acetylated. Sections were then hybridized for 18 h at 58°C, using a previously described hybridization solution (8). The next day, slides were washed in 2 \(\times\) standard saline citrate (SSC), washed in 0.1 \(\times\) SSC (58°C, 30 min), washed in 0.1 \(\times\) SSC (58°C, 60 min), and dehydrated through ascending concentrations of ethanol containing 6 M ammonium hydroxide.

Slides were apposed to Kodak SB5 film (Rochester, NY) to generate autoradiographic images. Emulsion autoradiographs were generated by dipping slides in Kodak NBT2 photographic emulsion. Emulsions were developed 2–4 wk later in Kodak D-19 developer (4 min), dipped in water, and then placed in Kodak Rapid fixer (4 min). Sections were counterstained with 0.5% toluidine blue, dehydrated in ethanol, placed in xylene, and coverslipped. Tissue sections were examined by light microscopy.

**Retroneuronal labeling of cardiac vagal afferents.** Cardiac vagal afferent neurons were identified with a fluorescent label, fluororuby (FR, 10,000-mol wt dextran-rodohamine conjugate, 10% solution, Molecular Probes, Eugene, OR), using the technique for selective retrograde labeling of cardiac vagal afferents as previously described (4). Briefly, after anesthesia with intraperitoneal methohexital sodium (50 mg/kg), adult male Sprague-Dawley rats (180–220 g) were intubated and mechanically ventilated (Harvard rodent respirator, model 683, Harvard Apparatus, South Natick, MA). Via left thoracotomy the heart was exteriorized, and the pericardium was closed with removal of air. After recovery from anesthesia, the heart was exteriorized, and the pericardium was removed. FR (16 \(\mu\)l) was injected into the base of the right and left ventricle in divided doses (4 \(\mu\)l each). The heart was then returned to its normal position, and the chest was closed with removal of air. After recovery from anesthesia, rats were extubated and returned to their cages. After a 2- to 3-wk survival time to allow retrograde axonal transport of dye to neuronal cell bodies, rats were euthanized and tissue was processed for immunocytochemistry or cellular electrophysiology as described below.

**Immunocytochemistry.** After a 2- to 3-wk survival time to allow retrograde axonal transport of FR to the neuronal cell bodies, rats were anesthetized with pentobarbital sodium perfused transcardially with 300 ml 0.1 phosphate-buffered saline (PBS), pH 7.4, and then 300 ml 4% paraformaldehyde in 0.1 M PBS, pH 7.4, at 4°C. Bilateral nodose ganglia were dissected and placed in 4% paraformaldehyde for 2 h and then transferred to 20% sucrose-PBS overnight at 4°C. Ganglia were embedded, and 10-\(\mu\)m sections were cut on a cryostat and thaw mounted on Superfrost/Plus slides. Sections were viewed with a Nikon microscope equipped with epifluorescence...
Solution changes were performed with the aid of a rapid extracellular solution-exchange device. This system permits >90% exchange of the bath surrounding the neuron in <1 s.

RESULTS

Radioreceptor assays. To examine adenosine receptor expression in the nodose ganglia, we first examined A₁ and A₂α adenosine receptor expression by radioreceptor assays. We tested for the presence of these receptor subtypes because functional studies suggest that adenosine receptors may be present on nodose ganglia (1, 6, 19) and selective radioligands for these subtypes are available.

Because of the small sizes of the ganglia, fixed doses of [³H]DPCPX (4 nM) or [³H]CGS-21680 (10 nM) were used, and assays were performed on crude tissue preparations. In the nodose ganglia, low-sensitivity [³H]DPCPX (a selective A₁ adenosine receptor ligand) binding was detectable (39.6 ± 1.2 fmol/mg protein), consistent with the presence of A₁ adenosine receptors. In contrast, we were not able to detect specific binding of [³H]CGS-21680 (a selective A₂α adenosine receptor ligand), although it was possible to detect specific labeling in crude brain tissue preparations (11.3 ± 4.2 fmol/mg protein).

As a complementary approach, we next directly examined A₁ and A₂α adenosine receptor expression by receptor-labeling autoradiography in tissue sections using [³H]DPCPX or [³H]CGS-21680. Similar to the results of radioreceptor assays, we could detect specific labeling using [³H]DPCPX in the nodose ganglia (Fig. 1). However, even with long exposures (6 mo), specific labeling of the ganglia using [³H]CGS-21680 was not detected, although labeling was detected in the striatum of rat brains with the same assay technique.

In situ hybridization. Nodose ganglia contain both neurons and nonneuronal cells, and it was not possible to localize labeling to specific cell types in receptor autoradiography studies. Therefore, in situ hybridization was next used to determine whether neurons identified visually expressed A₁ adenosine receptors. Analysis of emulsion autoradiographs revealed specific labeling in bilateral nodose ganglia (Fig. 2). Labeling was present over ~50% of neurons, but nonneuronal cells. There was no specific, organized distribution of labeled cells within the ganglia.

Colabeling studies. Immunocytochemistry and retrograde labeling. Autoradiographic and in situ hybridization experiments provided evidence that adenosine A₁ receptors were expressed on vagal afferent neurons. We next sought to determine whether adenosine A₁ receptors were present on cardiac vagal afferent neurons. Using colabeling studies, we identified neurons bearing the A₁ adenosine receptors using immunocytochemistry, and cardiac vagal afferent neurons were identified by retroneuronal labeling with FR. In retrograde labeling experiments, ~20% of sections had 1–2 FR-positive cells. FR-positive cells were present in bilateral nodose ganglia without a specific distribution. Of FR-positive neurons (cardiac vagal afferents), approximately one-half were strongly positive for A₁ adenosine receptors (Fig. 3).

Cellular electrophysiology. Because A₁ adenosine receptors were detected on a subset of vagal afferent neurons, including cardiac vagal afferents, we next sought to determine whether adenosine had functional electrophysiological effects in vagal afferent neurons. A total of 19 cells was studied. In patch-clamping experiments, the effects of adenosine, 2-chloro-N⁶-cyclopentyladenosine (CCPA; an A₁ selective agonist), 5'-N-ethylcarboxamidoadenosine (NECA; an A₂ selective agonist), and DPCPX (an A₁ selective antagonist), applied using a rapid solution exchanger, were tested on action potential configuration in current-clamp mode (n = 8) and on I_{Ca} in voltage-clamp mode (n = 11), using appropriate ionic substitution to isolate I_{Ca}. In a subset of experiments, cardiac afferents were identified by retrograde labeling.

Adenosine (10 µM) and CCPA (100 nM) had no measurable effect on action potentials under control conditions (Fig. 4, A and B). Application of the K⁺ channel blocker TEA prolongs the action potential and makes its duration sensitive to changes in the amplitude of I_{Ca}. Adenosine (10 µM) and CCPA (100 nM) significantly shortened, by 55 ± 22% (Fig. 4, C and D), action potentials that had been prolonged by the K⁺ channel blocker TEA in six of eight cells. This effect was blocked by DPCPX (5 µM). The A₂ adenosine receptor agonist NECA (10 µM) had no effect on action poten-
In the absence or presence of TEA (data not shown).

In the voltage-clamp mode, $I_{Ca}$ was completely blocked by 0.1 mM Cd (a nonspecific $I_{Ca}$ blocker) (Fig. 5A) and inhibited $32 \pm 9\%$ by 1 µM ω-conotoxin (a selective N-type calcium channel blocker) (Fig. 5B), consistent with the presence of N-type Ca channels. Adenosine and CCPA inhibited peak inward $I_{Ca}$ in 7 of 11 cells (Fig. 5C), including 2 of 3 labeled cardiac afferents, by $48 \pm 13\%$ without affecting voltage dependence (Fig. 5D). This effect was prevented by 5 µM DPCPX (Fig. 5E and F). NECA (10 µM) had no effect on $I_{Ca}$ (data not shown).

DISCUSSION

In this study, several complementary techniques, including radioreceptor assays, in situ hybridization, and immunocytochemistry, were used to provide the first direct evidence that adenosine receptors are present on a subset of vagal afferent neurons. The adenosine receptors identified were of the $A_1$ subtype; $A_{2a}$ adenosine receptors were not detectable on vagal afferents in our study. Furthermore, identification of cardiac vagal afferent neurons using retrograde transport of fluororuby, combined with immunocytochemical detection of $A_1$ adenosine receptors in colabeling studies, provide strong evidence that $A_1$ adenosine receptors are present on a subset of cardiac vagal afferent neurons. Finally, in patch-clamping experiments of vagal afferent neurons, including cardiac vagal afferents, adenosine reduced the inward $I_{Ca}$, demonstrating for the first time a direct functional action of adenosine on ionic currents in vagal afferent neurons, including cardiac vagal afferents.

There is evidence in humans that adenosine activates systemic reflexes originating in the heart (5). Intracoronary adenosine induces a reflex increase in blood pressure and plasma catecholamines in humans (5). In cardiac transplant recipients, in whom the heart is denervated, intracoronary adenosine does not produce these effects, supporting the concept that these effects are part of a reflex mediated by adenosine interaction with cardiac afferents (5). Shen and colleagues (19) demonstrated that intravenous adenosine produced vasovagal syncope in susceptible humans. These investigators speculated that adenosine interacts either directly or indirectly with cardiac vagal afferent neurons. Our data support the concept that...
adenosine directly interacts with cardiac vagal afferent neurons, potentially playing a role in the modulation of systemic reflexes.

Ischemic cardiac pain is believed to be mediated by sympathetic afferents, but not exclusively so. There is evidence that cardiac vagal afferents may also play a role in mediating cardiac pain (14). The mediator between cardiac ischemia and chest pain is unknown, but adenosine is a likely candidate. Studies in patients with stable angina have demonstrated that intracoronary adenosine produces angina-like chest pain in the absence of ischemia and that this chest pain is significantly diminished by the adenosine receptor antagonist aminophylline (6, 11). Gaspardone and colleagues (10) demonstrated that administration of the selective A1 receptor antagonist bamiphylline blocked pain generated by intracoronary adenosine administration in humans, providing evidence that cardiac pain is mediated by the A1 adenosine receptor. The present study lends support to the concept that adenosine may mediate cardiac pain, at least in part, by acting on cardiac vagal afferents. Studies in humans are consistent with our experimental findings, in which we found evidence of the A1 but not the A2a adenosine receptors in vagal afferent neurons, including those innervating the heart.

Armour and colleagues (1) demonstrated that, in anesthetized dogs, epicardial application of N6-cyclopentyladenosine enhanced spontaneous activity in 36 of 69 vagal afferent nerve fibers, consistent with a direct neuroexcitatory effect (1). To date, however, adenosine effects on individual ionic currents in vagal afferent neurons, specifically cardiac vagal afferent neurons, have not been investigated. In patch-clamped rodent dorsal root ganglion neurons (soma of sympathetic afferents), adenosine shortened the calcium-dependent portion of the action potential in a subset of sympathetic afferents (7, 13) and reduced a voltage-dependent $I_{Ca}$ in approximately one-half of the sympathetic afferents (7, 13). On the basis of affinity with selective agonists, either the A1 adenosine receptor (7) or a novel adenosine receptor (13) was thought to be the mediator. The organ specificity of this subset of afferents was not determined. Similar to the results in sympathetic afferents, we find that adenosine attenuates the voltage-dependent $I_{Ca}$ in a subset of vagal afferents, including cardiac vagal afferents. In addition, this effect was mediated through A1 adenosine receptors. Although adenosine’s action on $I_{Ca}$ did not alter the morphology of the action potential under control conditions, its effects on $I_{Ca}$ are likely to significantly modulate intracellular calcium dynamics in these neurons and by this mechanism to modulate neuroexcitability.

We hypothesize that the attenuation of $I_{Ca}$ by adenosine might render vagal afferent neurons more excitable by the following mechanism. Slow afterhyperpolarizations (AHPs) follow action potentials in a subset...
(≈50%) of vagal C afferent neurons, rendering them temporarily inexcitable (9). Attenuation of these slow AHPs increases the neuronal excitability. For example, bradykinin has been shown to inhibit slow AHPs on visceral afferents, thereby increasing their excitability (21). Slow AHPs are mediated by a calcium-dependent potassium current \( I_{K(Ca)} \). We speculate that adenosine similarly influences neuronal excitability in vagal afferents, specifically cardiac vagal afferents, by inhibiting calcium influx and thereby attenuating the \( I_{K(Ca)} \) and slow AHPs. This hypothesis is currently being tested in our laboratory.

Limitations. Because of the nature of our experiments, we focused our investigations on the neuronal cell body located in the nodose ganglia and not on the peripheral nerve endings in the myocardium. We assume that receptors present in the cell body are also likely to be present in the peripheral nerve terminals. Although we do not have definitive evidence to support this assumption, it is likely to be valid for several reasons. First, receptors sensitive to bradykinin, histamine, acetylcholine, and, most recently, mechanical stimulation have been localized to both the somata and the terminals of primary afferent neurons (8, 12). Second, several different groups have demonstrated that adenosine administered to the myocardium elicits systemic reflexes (5, 6, 10, 11).

The technique of retrograde labeling of cardiac vagal afferents does not label all cardiac vagal afferents. Therefore unlabeled cells may, or may not, innervate the heart. Cardiac vagal afferents are a heterogeneous group of neurons, including mechanosensitive, chemosensitive, and polymodal neurons. In our study we are unable to state definitely the proportion of cardiac
vagal afferents that have adenosine receptors. It is intriguing to note that not all vagal afferents identified as cardiac vagal afferents expressed the A₁ adenosine receptor. This is consistent with selectivity of function among cardiac vagal afferents, with only a subset mediating chemosensitivity to adenosine.

In summary, we provide radioreceptor, immunocytochemical, and functional evidence that adenosine A₁ receptors reside on a subset of vagal afferent neurons, including cardiac vagal afferents. The nodose ganglia receive sensory input from cardiac as well as noncardiac viscera. Because data from our in situ hybridization and patch-clamping experiments are consistent with adenosine receptors in up to 60% of nodose ganglion neurons, far more than innervate the heart, it is likely that adenosine receptors are present on sensory neurons innervating other (noncardiac) viscera as well. Adenosine released during ischemia of these viscera may also modulate systemic reflexes and mediate nociception, suggestive of a ubiquitous role for adenosine as a sensory neuromodulator.

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