Role of pulmonary C fibers in adenosine-induced respiratory inhibition in anesthetized rats

KEVIN KWONG, JU-LUN HONG, ROBERT F. MORTON, AND LU-YUAN LEE
Department of Physiology, University of Kentucky, Lexington, Kentucky 40536

Kwong, Kevin, J u-Lun Hong, Robert F. Morton, and Lu-Yuan Lee. Role of pulmonary C fibers in adenosine-induced respiratory inhibition in anesthetized rats. J. Appl. Physiol. 84(2): 417–424, 1998.—The clinical use of adenosine is commonly associated with pulmonary side effects, namely dyspnea, that suggest the possible involvement of bronchopulmonary sensory afferents. Our objective in this study was to characterize the effects of adenosine on breathing and to determine whether the vagal pulmonary afferents play a role in mediating these effects. We measured respiratory and cardiovascular changes in anesthetized, spontaneously breathing rats after bolus injections of adenosine at therapeutic doses. Right atrial injection of adenosine (0.04–0.6 mg/kg) elicits, in a dose-dependent manner, a pulmonary chemoreflex-like response consisting of a delayed apnea, bradycardia, and hypotension. In contrast, the classic capsaicin-elicted pulmonary chemoreflex occurs immediately after injection. Perineural capsaicin treatment of the cervical vagi blocked the adenosine-induced respiratory inhibition. Left ventricular administration of adenosine failed to elicit an apneic response. Pretreatment with the adenosine A1-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine attenuated the adenosine-induced apnea. These results indicate that adenosine elicits a respiratory inhibition via stimulation of pulmonary C fibers and that activation of the A1-receptor is probably involved. It is unclear, however, what accounts for the exceedingly long latency in this response.

dyspnea; vagus nerve; pulmonary chemoreflex; A1-receptor; 8-cyclopentyl-1,3-dipropylxanthine

ADENOSINE is a purine nucleoside product of ATP metabolism. It can be released by virtually any cell type in the body during conditions of insufficient oxygen supply. Its actions, through the various adenosine-receptor subtypes, bring about a decrease in energy demand and an increase in energy supply and are thus protective (2). For example, in ischemic cardiac muscles, adenosine is released and acts through the adenosine A1-receptor to decrease heart rate (HR) and atrioventricular (AV) nodal conduction (1). Both effects decrease the oxygen demand placed on the working muscles. It is these properties, in part, that make adenosine a widely used drug for the treatment of certain types of supraventricular tachycardia. However, there are certain commonly reported short-lived side effects associated with the therapeutic administration of adenosine: dyspnea, flushing, and chest discomfort (24). These sensations suggest the possible involvement of vagal sensory afferents.

The vagal bronchopulmonary afferent C fibers are small, unmyelinated nerve fibers that provide sensory input from airway and lung structures. The C-fiber nerve endings are believed to be located in proximity to the pulmonary capillaries and have also been found in the epithelium of conducting airways (4). Their stimulation either by inhaled irritants or by circulating auto- cytokids elicits prominent reflex bronchopulmonary responses including apnea, airway smooth muscle contraction, mucus hypersecretion, and extravasation of macromolecules in the tracheobronchial tree. Stimulation of these sensory fibers is also believed to be involved in evoking dyspneic sensations (9, 21, 29).

Because adenosine administration has been shown to elicit dyspneic sensations, it is reasonable to postulate that adenosine could elicit certain respiratory reflexes via stimulation of the vagal pulmonary C fibers. The objectives of this study were 1) to characterize the effects on breathing of adenosine at the therapeutic dose range, 2) to determine whether the vagal pulmonary C fibers play a role in mediating these respiratory effects, and 3) to determine which subtype(s) of the adenosine receptors is involved in the respiratory response.

METHODS

Surgical Procedures

Young male Sprague-Dawley rats (386 ± 9 g; n = 35) were anesthetized initially with injections of chloralose (100 mg/kg ip) and urethan (500 mg/kg ip), and supplemental doses of the same anesthetics were given intravenously (iv) to abolish the tail-pinch pain reflex. An iv line for later administration of various drugs was inserted into the jugular vein and was advanced until the tip of the catheter was close to the right atrium. The femoral artery was cannulated to measure arterial blood pressure (ABP) by using a pressure transducer (Statham P23AA). Body temperature was maintained at ~36°C with a servo-temperature controller and a heating pad placed under the animal. A short tracheal cannula was inserted just below the larynx via a tracheotomy, through which the animal breathed spontaneously in a supine position. Tracheal pressure was measured via a sidearm of the tracheal tube by a pressure transducer (Validyne MP45–28). Respiratory flow was measured with a heated pneumotachograph and a differential pressure transducer (Validyne MP45–14). The flow signal was integrated (Grass 7P10) to give tidal volume (VT). Ventilatory and ABP measurements were displayed on a polygraph (Grass model 7D), recorded on a multichannel tape recorder (HP 3968A; Hewlett-Packard), and digitized with an analog-to-digital converter (Keithley Metabyte) for online computer analysis (TS-100 series software; Biocybernetics). Duration of inspiration and expiration, respiratory frequency, VT, minute ventilation, and HR were all analyzed on a breath-by-breath basis. Results obtained from the computer analysis were routinely compared for accuracy with results obtained by hand calculation.

Chemicals

Stock solution of adenosine hemisulfate salt (Sigma Chemical, St. Louis, MO) was dissolved in isotonic saline. Stock solution of capsaicin (Sigma) was prepared in a vehicle of 10% Tween 80, 10% ethanol, and 80% isotonic saline. Stock solution of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX;
Sigma) and N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine (DPMA; Research Biochemicals International, Natick, MA) was prepared in a vehicle of 100% dimethyl sulfoxide (DMSO; Sigma). Stock solution of 3,7-dimethyl-1-propargylxanthine (DMPX; Research Biochemicals International) was dissolved in saline at a concentration of 0.8 mg/ml. Solutions of desired concentration based on the animal's body weight were prepared daily with saline dilution. The volume of each bolus injection was 0.15 ml, which was then flushed from the catheter (dead space = 0.3 ml) into the circulation by an injection of 0.45 ml saline. Before each challenge, the lungs were hyperinflated (tracheal pressure >10 cmH_2O) to establish a constant volume history.

Experimental Protocols

Four series of experiments were carried out. The specific experimental procedures are as follows.

Protocol 1: Dose-dependent effect of adenosine on respiration. To determine whether the therapeutic dosage of adenosine induces a cardiorespiratory response in a dose-dependent manner, we carried out this series of experiments in six rats. We chose the 0.04 (low dose), 0.15 (middle dose), and 0.6 mg/kg (high dose) concentrations to mimic the dosage of adenosine for its clinical use (5, 24). The sequence of adenosine injections was alternated to achieve a balanced design. The reproducibility of the cardiorespiratory response to adenosine was established in all rats tested. Injections in this and all subsequent protocols were made with a minimum of 10-min intervals between injections, although no tachyphylaxis was observed in our preliminary trials with injections at 5-min intervals.

Protocol 2: Effect of perineural capsaicin treatment of the vagi. To determine whether the reflex response to adenosine is elicited by the stimulation of vagal afferent C fibers, we selectively and reversibly blocked C-fiber conduction through both the left and right vagi in seven rats. The procedure is a modification of the one originally demonstrated by Jancsó and Such (13) and has been used successfully in our laboratory (17). Briefly, having isolated both cervical vagi, we wrapped capsaicin-soaked (150–450 µg/ml) cotton swabs around a 2- to 3-mm segment of the nerves for 10–20 min; then we removed the swabs and irrigated the pocket with isotonic saline. The success of the perineural capsaicin-treatment procedure was judged on the basis of two criteria: 1) the abolition of the reflex induced by injections of capsaicin (0.5–1.0 µg/kg), a known potent stimulator of pulmonary C fibers, and 2) no change of the Hering-Breuer inflation reflex (tracheal pressure 5–7 cmH_2O). Both criteria imply that the myelinated fibers were largely unaffected and that the blockade was therefore selective for the unmyelinated fibers.

Protocol 3: Right atrial vs. left ventricular injection. To determine the location of the afferent C-fiber endings involved in eliciting the apneic response, we compared responses to left ventricular and right atrial injections of adenosine in seven rats. Adenosine injected into the left ventricle bypasses the pulmonary circulation and presumably does not have immediate access to the pulmonary C fibers. For the left ventricular injections, we cannulated the right carotid artery with a blunt-tipped catheter and advanced the tip retrogradely through the brachiocephalic artery, through the ascending aorta, past the aortic valve, and finally into the left ventricle. The placement of the tip of the
The respiratory response to adenosine involves the activation of the A2-receptor, we compared the responses to adenosine before and ~10 min after infusion of DMPX (0.1 mg·kg⁻¹·min⁻¹ for 10 min), a specific antagonist of the A2-receptor, in seven other rats. This dose of DMPX has been shown to effectively block A2-receptors in vivo (28). In addition, as a positive control, we tested the effectiveness of this dose of DMPX in antagonizing the hypotensive effects of DPMA, a selective A2-receptor agonist, in six of these rats (15). A syringe pump (World Precision Instruments SP 100i) was used to infuse each antagonist into the animal via the right femoral vein.

Statistics

Data were reduced for statistical analysis in the following manner. The respective baseline values were averaged over the 10 breaths immediately before injection. Minute ventilation values were obtained by averaging the three lowest consecutive values within 10 breaths after the injection of adenosine. Because of the moderately long duration of the hypotension, the lowest mean arterial blood pressure (MABP) values after injection were averaged over a period of 20 breaths. Because of the transient nature of adenosine-induced bradycardia, the lowest HR values were averaged over a period of three breaths. Results were then analyzed by a two-way repeated-measures analysis of variance. When a positive interaction was detected, pairwise comparisons were made by using Fisher’s least significant difference. P values < 0.05 were considered significant. Data are expressed as means ± SE.

RESULTS

Protocol 1: Adenosine Induces a Pulmonary Chemoreflexlike Response in a Dose-Dependent Manner

Adenosine elicited a ventilatory inhibition in a dose-dependent manner, with the high dose producing the strongest inhibition. Each event occurred with a latency of ~5 s (Fig. 1). Respiration quickly returned to baseline with the middle- and low-dose injections, but with the high dose, there was a slight but noticeable increase in respiration ~20 s after the injection. The bradycardia and hypotension displayed the same dose-dependent relationship but with an onset latency of ~3 s.

When values for five rats were averaged, the baseline minute ventilation remained stable across all three dosages (Fig. 2). Each dose of adenosine elicited a significant dose-dependent decrease in minute ventilation that was also significantly different from each other. On average, minute ventilation started to decrease at about four breaths after the injection of each dose of adenosine, and the duration of this inhibitory effect lasted about five breaths. The transient depression in minute ventilation (28.6 ± 3.7 ml/min) induced by the high dose of adenosine was followed by a significant increase in minute ventilation (100.1 ± 9.6 ml/min) with respect to baseline (78.0 ± 5.5 ml/min), resulting mainly from an increase in Vt. The adenosine-induced bradycardia and hypotension in each of the three dose levels were significantly different from their respective baselines and from each other. Saline injection produced no ventilatory response in this or any subsequent protocols. The bolus injection of saline did cause a transient bradycardia, but only in the first 2 s.
Protocol 2: Perineural Capsaicin Treatment of the Vagi Abolishes the Adenosine-Induced Inhibition of Breathing

The capsaicin-induced apnea (1.0 µg/kg) was abolished by the perineural capsaicin treatment of the vagi, verifying its effectiveness in blocking the vagal afferent C-fiber-mediated reflex response (Fig. 3, A and C). The adenosine-induced ventilatory depression (0.3 mg/kg) was similarly abolished after perineural treatment and recovered in ~1 h (Fig. 3, B, D, and F). The perineural treatment did not eliminate but did attenuate the adenosine-induced bradycardia and hypotension (Fig. 3, B and D).

When values for seven rats were averaged, the baseline minute ventilation did not change after perineural capsaicin treatment (122.3 ± 7.4 ml/min, Fig. 4). Before perineural treatment, injection of adenosine produced a significant decrease (64.2 ± 3.8 ml/min) and then a subsequent increase in minute ventilation (136.5 ± 9.4 ml/min). After completion of the treatment, injection of adenosine failed to elicit a significant depression in minute ventilation, but it did induce a subsequent increase in minute ventilation (138.0 ± 5.7 ml/min). The baseline MABP was slightly but significantly elevated after perineural treatment. Adenosine induced a significant hypotension before and after treatment. There was no change in the adenosine-induced bradycardia before and after perineural treatment. In two rats, sham perineural treatment of the vagi, using isotonic saline-soaked cotton swabs, produced no effect on the adenosine-induced cardiopulmonary responses.

Protocol 3: Adenosine Fails To Elicit an Apneic Response When Injected Into the Left Ventricle

Whereas the right atrial injection of adenosine (0.6 mg/kg) produced the same pattern of ventilatory depression, as described above, the left ventricular injection failed to elicit an apnea (Fig. 5). Although adenosine injected into the right atrium induced bradycardia and hypotension with an ~3-s onset latency, adenosine injected into the left ventricle induced similar effects but with only an ~1-s onset latency.

When values for seven rats were averaged, right atrial injection of adenosine initially depressed minute ventilation (69.8 ± 16.9 ml/min), but left ventricular injection of adenosine failed to elicit any significant ventilatory depression (Fig. 6). However, both left- and right-heart injections induced significant increases in minute ventilation (156.8 ± 31.3 and 156.5 ± 25.4 ml/min, respectively) ~20–30 breaths after injection. The increases were not significantly different from each other. There was no significant difference in the magnitude of cardiovascular response when adenosine was administered into the left ventricle compared with its administration into the right atrium.

Protocol 4: DPCPX Significantly Reduces the Adenosine-Induced Apneic Response

The A1-receptor antagonist DPCPX abolished the adenosine-induced bradycardia, demonstrating the effectiveness of the antagonist treatment (Fig. 7). DPCPX nearly abolished the adenosine-elicited respiratory inhibition and the subsequent increase in minute ventilation.

When values for eight rats were averaged, there was no significant change in baseline minute ventilation after infusion of DPCPX (Fig. 8). The DPCPX treatment significantly attenuated but did not abolish the adenosine-induced ventilatory depression (85.9 ± 9.2 vs. 49.9 ± 5.5 ml/min, before vs. after DPCPX, respectively). Although the DPCPX treatment abolished the adenosine-induced bradycardia, it did not affect the adenosine-induced hypotension. The immediate bradycardia at adenosine injection after DPCPX pretreat-
ment reflects an effect caused by a rapid bolus injection; the same bradycardia also appears after a rapid bolus injection of saline. Infusion of the vehicle DMSO into six rats produced no change in the baseline or cardiopulmonary responses to adenosine. No similar attempt was made in the remaining two rats. In seven other rats, a similar protocol was followed to test whether the A2-receptor antagonist DMPX had any effect on the cardiorespiratory parameters. DMPX pretreatment did not affect the adenosine-induced ventilatory depression ($P > 0.05; n = 7$) but did attenuate the adenosine-induced hypotension ($60.3 \pm 1.7$ vs. $71.8 \pm 2.2$ mmHg, before vs. after DMPX, respectively) as well as the DPMA-induced hypotension ($75.3 \pm 3.0$ vs. $95.0 \pm 4.9$ mmHg, before vs. after DMPX, respectively).

**DISCUSSION**

Our results show that iv injection of adenosine in the range of therapeutic doses acutely inhibits respiration via stimulation of vagal pulmonary C fibers. This reflex inhibitory response occurs in a dose-dependent manner, involves the A1-receptor subtype, and exhibits an onset latency of 5–7 s. As expected, administration of adenosine also causes bradycardia and hypotension. Although vagotomy abolished the acute adenosine-induced ventilatory inhibition (data not shown), we employed perineural capsaicin treatment of the vagi, which selectively blocks conduction through the small unmyelinated vagal C fibers. Our data show that this bilateral blockade of vagal C-fiber conduction abolished the adenosine-mediated reflex respiratory inhibition (Figs. 3 and 4). The vagal C-fiber endings are presumably located in lung structures that receive blood perfusion from the pulmonary circulation (lung parenchyma, intrapulmonary airways, and others) (27), because the left ventricular injection of adenosine, which prevents immediate access of the injected adenosine to the pulmonary circulation, failed to elicit a ventilatory inhibition (Figs. 5 and 6). The A1-receptor antagonist DPCPX significantly suppressed the adenosine-induced respiratory inhibition (Figs. 7 and 8), whereas neither the vehicle DMSO nor the A2-receptor antagonist DMPX exhibited any effect on the adenosine-induced respiratory inhibition. The selectivity of DPCPX can be inferred by its potency in antagonizing the bradycardia, which is mediated through the A1-receptor, but not the hypotension, which is mediated through the A2-receptor (2, 14). The selectivity of DMPX can be inferred by its potency in antagonizing the DPMA-induced hypotension (15).

Although this adenosine-induced cardiorespiratory response resembles the triad of the classic pulmonary responses to RA injection and left ventricular (LV) injection of adenosine in anesthetized, spontaneously breathing rat (373 g). A: responses to RA injection of adenosine (0.6 mg/kg). B: responses to LV injection of the same dose of adenosine. See Fig. 1 for details.
chemoreflex (apnea, bradycardia, and hypotension), such as the one elicited by iv administration of capsaicin (4), important differences exist between the two responses. The adenosine-induced bradycardia and hypotension are not solely a vagally mediated reflex response; there was no difference in the adenosine-induced cardiovascular effects before and after perineural capsaicin treatment of the vagi (Figs. 3 and 4). However, we cannot rule out the possibility of a cardiovascular component in this reflex. Although the bradycardia is statistically indistinguishable before and after the perineural treatment, there was a noticeable decrease in three of the seven animals, suggesting that the perineural treatment attenuated a reflex bradycardia. Previous investigators have reported that the adenosine-induced bradycardia and hypotension are elicited primarily by a direct modulation of the AV node to decrease its conduction, of the sinoatrial node to decrease HR, and of the atrial myocytes to decrease contractility (1). These effects result in a decreased cardiac output, which can decrease the ABP. Adenosine can also directly cause vascular smooth muscle relaxation to decrease ABP via the A2 receptor (2). Although the bradycardia and hypotension consistently precede the apnea, they are not the cause of the apnea. Left ventricular injection of adenosine elicits a strong bradycardia and hypotension without eliciting an apnea (Figs. 5 and 6). Furthermore, the time course of the adenosine-elicited reflex ventilatory inhibition exhibits a longer latency of onset than the capsaicin-elicited reflex ventilatory inhibition (Fig. 3).

Clinically, adenosine is widely used for the treatment of paroxysmal supraventricular tachycardia (PSVT), but complaints of dyspneic sensations are frequently reported (24). PSVT is usually caused by a reentrant circuit that involves the AV node (24). Slowing the electrical conduction through this node is the mechanism by which adenosine converts PSVT to sinus rhythm (6). The therapeutic dosage for adenosine is independent of body weight for adults: the initial dose is 6 mg, followed by a second dose of 12 mg if the initial dose is ineffective (24). In children, however, the dosage of adenosine is based on weight: 0.05–0.25 mg/kg (5). The method of administration requires that adenosine be rapidly injected as a bolus (within 2 s) into a large peripheral vein and then be followed by a saline flush. Accordingly, our dose-response protocol was chosen to mimic these clinical dosages. In our study, adenosine exhibits great variability between animals in the cardiovascular response, thus confirming clinical observations (7, 25). We find that the same dose of adenosine also elicits a respiratory response that exhibits a similar, if not greater, variability.

![Figure 6](http://example.com/fig6.png)

**Fig. 6.** Group data (means ± SE) comparing responses to RA and LV injections of adenosine in 7 anesthetized, spontaneously breathing rats. ○, RA injections of adenosine (0.3–0.6 mg/kg); ●, LV injections of adenosine (0.3–0.6 mg/kg). See Fig. 2 for details.

![Figure 7](http://example.com/fig7.png)

**Fig. 7.** Experimental record illustrating effects of A1-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on adenosine-induced cardiorespiratory response in anesthetized, spontaneously breathing rat (364 g). A: responses to RA injection of adenosine (0.3 mg/kg) before DPCPX. B: responses to injection of same dose of adenosine 10 min after DPCPX infusion (10 µg·kg⁻¹·min⁻¹ for 10 min). See Fig. 1 for details.
There has been at least one instance of a premature human infant who exhibited a long apnea when adenosine was administered (5). Some studies in rats have shown that adenosine analogs act centrally to depress respiration (30). These studies showed that cerebral intraventricular injection of adenosine analogs depressed ventilation. In addition, endogenously released adenosine may be involved in mediating the hypoxic depression of ventilation in rabbit pups (26) and in adult cats (18). The present study, using iv bolus injections of adenosine, shows an acute ventilatory inhibition. Although we cannot discount the fact that the central effects of adenosine may play a role in the ventilatory inhibition that we are reporting, our data clearly show that a vagal reflex is involved. Perineural capsaicin treatment of the vagi abolished the respiratory inhibition. Moreover, a recent study in our laboratory (12) has established the first direct electrophysiological evidence of the stimulatory effect of adenosine, administered at the same dosages as that in this study, on pulmonary afferent C fibers in rats. However, Pelleg and Hurt (22) tested right atrial injection of adenosine in dogs and failed to find a stimulatory effect on pulmonary C fibers. We can only make conjectures regarding the discrepancy between these two studies, as their study did not include the data necessary to make meaningful comparisons; however, a probable explanation could simply be a species difference.

Previous studies have shown that ventilation increases in humans with adenosine infusion (8) and in rats with adenosine analogs by stimulation of the carotid bodies (19). The latter study describes a ventilatory increase that lasts for 30–50 s after injections of adenosine or its analogs into the common carotid artery. Our data show that adenosine injected into either the right atrium or left ventricle resulted in increased respiration, starting 15–20 s after administration of the drug. The hyperpnea induced by adenosine was not affected by vagal C-fiber conduction blockade with perineural capsaicin treatment of the vagi, suggesting that the acute respiratory inhibition elicited by the stimulation of vagal pulmonary C fibers by adenosine is distinctly different from the hyperpneic response presumably elicted by the stimulation of the carotid bodies.

The respiratory inhibition occurs with an onset latency of 5–7 s after the injection of adenosine. Although we do not fully understand the cause of such a long delay, the possible involvement of certain mechanisms should be considered. The signal transduction of adenosine is mediated through an adenosine 3',5'-cyclic monophosphate (cAMP)-independent or a cAMP-dependent mechanism, depending on the receptor subtype (20). The cAMP-dependent pathway works through a trimeric G-protein-coupled receptor that can be linked to a wide variety of downstream effectors, including adenyl cyclase, inositol 1,4,5-trisphosphate, calcium channels, and potassium channels (20). In contrast, capsaicin activates a putative capsaicin receptor that is thought to be coupled directly to a nonselective cation channel, the opening of which leads to the depolarization of the nerve terminal (11). Presumably, a receptor directly coupled to an ion channel would respond faster to stimuli than would a G-protein-coupled receptor (10). In fact, prostaglandins, the receptors of which are supposedly G-protein-coupled (3), also stimulate C-fiber endings with a similar long-onset latency (4, 16). Alternatively, the A1-receptors may not reside on the C-fiber nerve ending but rather on another cell type (e.g., mast cells) (23). On stimulation of the A1-receptor by adenosine, the cell will release mediators that subsequently stimulate the C-fiber endings.

In conclusion, we have demonstrated an inhibitory ventilatory effect of adenosine administered at therapeutic doses, and the effect is elicited by the stimulation
of vagal pulmonary C fibers. This C-fiber stimulation involves the activation of the $A_1$-receptor and may be responsible for the dyspneic side effect related to the clinical administration of adenosine.

The authors thank Dr. Mary K. Rayens for statistical analysis of the data and J enny L. Farley for technical assistance in preparing this manuscript.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-40369 and by Grant 5–41066 from the Kentucky Tobacco and Health Research Board.

Address for reprint requests: L.-Y. Lee, Dept. of Physiology, Chandler Medical Center, University of Kentucky, Lexington, KY 40536–0084 (E-mail: lylee@pop.uky.edu).

Received 6 May 1997; accepted in final form 29 August 1997.

REFERENCES


