Epinephrine enhances the sensitivity of rat vagal chemosensitive neurons: role of \( \beta_3 \)-adrenoceptor

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Gu Q, Lin Y-S, Lee L-Y. Epinephrine enhances the sensitivity of rat vagal chemosensitive neurons: role of \( \beta_3 \)-adrenoceptor. J Appl Physiol 102: 1545–1555, 2007. First published December 14, 2006; doi:10.1152/japplphysiol.01010.2006.—This study was carried out to determine whether epinephrine alters the sensitivity of rat vagal sensory neurons. In anesthetized rats, inhalation of epinephrine aerosol (1 and 5 mg/ml, 3 min) induced an elevated baseline activity of pulmonary C fibers and enhanced their responses to lung inflation (20 cmH\(_2\)O, 10 s) and right atrial injection of capsaicin (0.5 \( \mu \)g/kg). In isolated rat nodose and jugular ganglion neurons, perfusion of epinephrine (3 \( \mu \)M, 5 min) alone did not produce any detectable change of the intracellular Ca\(^{2+} \) concentration. However, immediately after the pretreatment with epinephrine, the Ca\(^{2+} \) transients evoked by chemical stimuli (capsaicin, KCl, and ATP) were markedly potentiated; for example, capsaicin (50 nM, 15 s)-evoked Ca\(^{2+} \) transient was increased by 106% after epinephrine (\( P < 0.05; n = 11 \)). The effect of epinephrine was mimicked by either BRL 37344 (5 \( \mu \)M, 5 min) or ICI 215,001 (5 \( \mu \)M, 5 min), two selective \( \beta_3 \)-adrenoceptor agonists, and blocked by SR 59230A (5 \( \mu \)M, 10 min), a selective \( \beta_3 \)-adrenoceptor antagonist, whereas pretreatment with phenylephrine (\( \alpha_1 \)-adrenoceptor agonist), guanabenz (\( \alpha_2 \)-adrenoceptor agonist), dobutamine (\( \beta_1 \)-adrenoceptor agonist), or salbutamol (\( \beta_2 \)-adrenoceptor agonist) had no significant effect on capsaicin-evoked Ca\(^{2+} \) transient. Furthermore, pretreatment with SQ 22536 (100–300 \( \mu \)M, 15 min), an adenylyl cyclase inhibitor, and H89 (3 \( \mu \)M, 15 min), a PKA inhibitor, completely abolished the potentiating effect of epinephrine. Our results suggest that epinephrine enhances the excitability of rat vagal chemosensitive neurons. This sensitizing effect of epinephrine is likely mediated through the activation of \( \beta_3 \)-adrenoceptor and intracellular cAMP-PKA signaling cascade.

stress; vagal afferents; fura-2; adenylyl cyclase; cAMP-dependent protein kinase

EPINEPHRINE IS THE PRIMARY CATECHOLAMINE secreted by the adrenal medulla in response to various physiological stresses (41). The physiological functions of epinephrine are mediated through the activation of cell membrane \( \alpha \)- and \( \beta \)-adrenoceptors, both of which belong to the superfamilies of seven-transmembrane G-protein-coupled receptors (13). Vagus nerves provide the primary afferent innervation of a broad spectrum of visceral organs and play an important role in the initiation of visceral/visceroceptive reflexes and the regulation of vegetative functions (24, 29). Numerous studies have identified the presence of \( \beta \)-adrenoceptors on the vagus nerve in both rats and humans (23, 28). Although a sensitizing effect of epinephrine on nociceptors was recently reported (21), whether epinephrine can modulate the excitability of vagal sensory neurons is largely unknown.

The aims of the present study were 1) to determine whether epinephrine alters the excitability of pulmonary vagal afferents in vivo and the chemosensitivity of isolated vagal sensory neurons in vitro; 2) if so, to identify the specific subtype of adrenoceptors subserving the effect of epinephrine; and 3) to investigate the involvement of cAMP-PKA intracellular transduction cascade in mediating this effect. Since intracellular Ca\(^{2+} \) is known to play a critical role in regulating a diverse range of cellular processes such as neuronal membrane excitability, neurotransmitter release, synaptic plasticity, cell proliferation, and gene transcription (3), fura-2-based ratiometric Ca\(^{2+} \) imaging was employed to determine the sensitivity of these vagal sensory (nodose and jugular ganglion) neurons isolated from adult Sprague-Dawley rats. Three chemical agents (capsaicin, KCI, and ATP) known to stimulate vagal sensory neurons (12) by activating different ion channels were used as the chemical stimuli in the Ca\(^{2+} \) imaging study.

METHODS

The procedures described below were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and were also approved by the University of Kentucky Institutional Animal Care and Use Committee.

In Vivo Study

Recording of single-fiber activity of pulmonary afferents. Sprague-Dawley rats (280–455 g; \( n = 31 \)) were initially anesthetized with an intraperitoneal injection of \( \alpha \)-chloralose (100 mg/kg) and urethane (500 mg/kg) dissolved in a borax solution (2%); supplemental doses of these anesthetics were injected intravenously to maintain abolition of pain reflexes. A short tracheal cannula was inserted after a tracheotomy, and tracheal pressure was measured (Validyne MP 45-28, Northridge, CA) via a side port of the tracheal cannula. The expiratory outlet of the respirator was placed under 3 cmH\(_2\)O pressure to maintain a near-normal functional residual capacity. Tidal volume and frequency were set at 8–10 ml/kg and 50 breaths/min, respectively. One femoral artery was cannulated for recording the arterial blood pressure (ABP). For right-atrial injection of capsaicin, the left jugular vein was cannulated, and a catheter was advanced until its tip was positioned just above the right atrium. The volume of each bolus injection was 0.15 ml, which was first injected into the catheter (dead space, \( \sim 0.2 \) ml) and then flushed into the circulation by an injection of 0.4 ml of saline. Body temperature was maintained at \( \sim 36 \)°C by means of a heating pad placed under the animal. At the end of the experiment, the animal was killed by an intravenous injection of KCI.

Vagal pulmonary afferents can be broadly classified into three major types, based on the criteria established by previous investigators (33): slowly adapting receptors (SARs), rapidly adapting receptors

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(RAs), and pulmonary C-fibers. These pulmonary afferents were identified using the procedures described previously (14), and the fiber activity (FA) was measured by the conventional method of single-fiber recording. Briefly, right cervical vagus nerve was sectioned as far rostrally as possible, and the caudal end of the cut vagus was placed on a small dissecting platform and immersed in a pool of mineral oil. A thin filament was teased away from the desheathed nerve trunk and placed on a miniature platinum-iridium electrode. Action potentials were amplified, monitored by an audio monitor, and displayed on an oscilloscope. The thin filament was further split until the action potentials arising from a single unit were electrically isolated. FA, tracheal pressure, ABP, and heart rate were analyzed to determine the single-unit responses to lung inflation with an online data acquisition system (Biocybernetics TS-100, Taipei, Taiwan).

**Experimental protocols.** To study the effect of epinephrine on pulmonary C-fiber afferents, the single-unit responses to lung inflation (20 cmH2O, 10 s) and to capsaicin (0.5 μM) were investigated before and 2, 10, and 20 min after the administration of epinephrine. To minimize systemic effects, epinephrine was delivered into the airways by aerosol; during the delivery of the epinephrine, the ultrasonic nebulizer (Lumiscope 6610; Lumiscope, East Brunswick, NJ) that generated the aerosol was connected between the outlet of the respirator and the tracheal tube to prevent contamination of the breathing circuit by the residual epinephrine. The mass mean diameter of aerosol and the total volume of solution delivered over 3 min under our experimental setting were estimated to be 3–4 μm and 0.05–0.06 ml, respectively. To determine whether the effect of epinephrine was dose dependent, three doses of epinephrine were used in different groups of rats: 0 mg/ml (vehicle: 5 mg/ml ascorbic acid in saline), 1 mg/ml (low concentration), and 5 mg/ml (high concentration). No more than two doses of epinephrine were administered in each animal. Because of the long-lasting effect of the high concentration of epinephrine, responses were also measured at 60 min after the aerosol challenge. Identical protocols were followed to study the effect of epinephrine on the responses of SAs and RAs to lung inflation (20 cmH2O, 10 s) in a separate group of rats.

**In Vitro Study**

Isolation and culture of nodose and jugular ganglion neurons. Because subtypes of adrenergic receptors are known to be expressed in different cell types in the airways and their individual involvements cannot be differentiated in the in vivo preparation, the following experiments were performed in isolated vagal sensory neurons to identify the receptor subtypes subserving the effect of epinephrine. Young adult male Sprague-Dawley rats (150–220 g; n = 35) were anesthetized with 4% halothane and decapitated. The head was immediately immersed in ice-cold Hank’s balanced salt solution. Nodose and jugular ganglia were extracted under a dissecting microscope and placed in ice-cold Dulbecco’s minimal essential medium/F-12 (DMEM/F12) solution. Each ganglion was desheathed, cut into ~10 pieces, placed in 0.125% type IV collagenase, and incubated for 1 h in 5% CO2 in air at 37°C. The ganglion suspension was centrifuged (150 g, 5 min) and supernatant aspirated. The cell pellet was resuspended in 0.05% trypsin in Hank’s balanced salt solution for 5 min and centrifuged (150 g, 5 min); the pellet was then resuspended in a modified DMEM/F12 solution [DMEM/F12 supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 100 μM MEM nonessential amino acids] and gently triturated with a small bore fire-polished Pasteur pipette. The dispersed cell suspension was centrifuged (500 g, 8 min) through a layer of 15% bovine serum albumin to separate the cells from the myelin debris. The pellets were resuspended in the modified DMEM/F12 solution supplemented with 50 ng/ml 2.5S nerve growth factor, plated onto poly-L-lysine-coated glass coverslips, and then incubated overnight (5% CO2 in air at 37°C).

In a subset of experiments, sensory neurons innervating the lungs and airways were identified by retrograde labeling from the lungs (22) with the fluorescent neuronal tracer, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiI). Briefly, rats (140–180 g) were anesthetized with intraperitoneal injection of pentobarbital sodium (40 mg/kg) and intubated with a polyethylene catheter (PE-150) with its tip positioned in the trachea above the thoracic inlet. DiI was initially sonicated and dissolved in ethanol, diluted in saline (1% ethanol, vol/vol), and then instilled into the lungs (0.2 mg/ml; 0.2 ml × 2) with animal’s head tilted upward at ~30°C. The animal was allowed to recover for 7–10 days to permit DiI to be transported back to the cell soma of pulmonary vagal sensory neurons.

**Intracellular Ca2+ measurement.** Intracellular Ca2+ was monitored using the fluorescent Ca2+ indicator fura 2-AM. Cells were loaded with 5 μM fura 2-AM for 30 min at 37°C, then rinsed (×3) with extracellular solution and allowed to deesterify for at least 30 min before use. Ratiometric Ca2+ imaging was performed using a fluorescence inverted microscope (Axiovert 100; Carl Zeiss, Thornwood, NY) equipped with a variable filter wheel (Lambda 10-2; Sutter Instruments, Novato, CA) and a digital charge-coupled device camera (Princeton Instruments, Trenton, NJ). Dual images (340- and 380-nm excitation, 510-nm emission) were collected and pseudocolor ratio-metric images monitored during the experiments by using the software Axios Imaging Workbench (Axon Instruments, Union City, CA). The imaging system was standardized with a two-point calibration, using a Ca2+-free standard (−) and a Ca2+-saturated standard (+). Both standards contained 11 μM fura 2 [4 μl of 10 mM fura 2 Penta K+ salt, 8 ml of 20 mM HEPES-Na (pH 7.4), 32 ml of H2O] and were prepared as follows: (− standard) 18 ml of fura 2, 1.98 ml of 10 mM EGTA-Na (pH 7.6); (+ standard) 18 ml fura 2, 1.98 ml of 10 mM CaCl2. The parameters used for the two-point calibration include: the dissociation constant of fura-2 (Kd; 225), the ratio values for the − and + concentration standards (Rmin and Rmax), and the denominator wavelength intensities for the − and + concentration standards (Denmin and Denmax). The intracellular Ca2+ concentration ([Ca2+]i) in nM was estimated according to the following equation described by Grynkiewicz et al. (11): [Ca2+]i = Kd[(R − Rmin)/(Rmax − R)](Denmax/Denmin). Typical Rmin and Rmax values were 0.586 and 2.54, respectively.

**Experimental protocols.** Following the incubation period with fura 2-AM, the coverslip containing vagal sensory neurons was mounted onto a chamber (0.2 ml) placed on the stage of the microscope. During the experiments, the entire chamber was continuously perfused with the standard extracellular solution (containing in mM: 5.4 KCl, 136 NaCl, 1 MgCl2, 1.8 CaCl2, 0.33 NaH2PO4, 10 glucose, 10 HEPES; pH 7.4) or with standard extracellular solution containing various pharmacological and chemical agents by a gravity-fed valve-control system (VC-66CS; Warner Instruments, Hamden, CT). At the onset of each test, the perfusing solution was switched from one to another (e.g., from extracellular solution to epinephrine or from epinephrine to capsaicin) and delivered via the common outlet of a manifold operated by the valve-control system. The perfusion was kept at a constant rate of ~2 ml/min; a complete change of bath solution occurred in 6 s. KCl solution (100 mM) was perfused at the end of each experimental run to test for cell viability. All experiments were performed at room temperature (20–23°C).

Three study series were performed to determine: 1) the effect of epinephrine on the Ca2+ transients evoked by low doses of chemical stimulants including capsaicin, KCl, and ATP; 2) the adrenergic subtype(s) potentially involved in the effect of epinephrine; and 3) the possibility of the involvement of cAMP-PKA transduction cascade.

**Chemicals.** DMEM/F12, trypsin and 2.5S-nerve growth factor were obtained from Invitrogen (Calsbad, CA). Fura 2-AM and DiI were prepared as follows: (1) fura 2-AM (110 μM) was perfused at the end of each experimental run to test for cell viability. All experiments were performed at room temperature (20–23°C).
(β2-adrenoceptor agonist), BRL 37344 (β1-adrenoceptor agonist), ICI 215,001 (β1-adrenoceptor agonist), SR 59230A (β2-adrenoceptor antagonist), SQ 22536 (adenylate cyclase inhibitor), and H89 (PKA inhibitor) were obtained from Sigma (St. Louis, MO).

In the Ca2+ imaging study, stock solution of capsaicin (1 mM) was prepared in a vehicle of 10% Tween 80, 10% ethanol, and 80% extracellular solution; H89 (10 mM) was dissolved in dimethyl sulphoxide; epinephrine (20 mM) was dissolved in a mixture of extracellular solution with equivalent amount of ascorbic acid before each experiment and was kept on ice in subdued lighting conditions. These stock solutions were then diluted with the extracellular solution to yield the appropriate concentration before application. All other chemicals were prepared daily in extracellular solution before applications. The concentrations of BRL 37344 and SR 59230A used have been reported to effectively and selectively activate and antagonize, respectively, the rat β2-adrenoceptors (18). The concentration of ICI 215,001 was selected based on a concentration-response relationship demonstrating that this concentration produces ~75% of β2-adrenoceptor-mediated maximal relaxing effect in guinea pig ileum strips while showing no significant agonist activities on guinea pig atrium β1- and trachea β2-adrenoceptors (39). The concentrations of SQ 22536 and H89 used have been shown to be effective in selectively inhibiting adenylate cyclase and PKA, respectively, in various cell types (12, 34).

In the single-fiber recording study, desired concentrations of the pharmacological agents were prepared in a similar manner, except that isotonic saline, instead of extracellular solution, was used as vehicle.

**Statistical Analysis**

A one- or two-way repeated-measures ANOVA was used for the statistical analysis. When results of the ANOVA showed a significant interaction, pairwise comparisons were made with a post hoc analysis (Newman-Keuls test). Data are reported as means ± SE. A P value of <0.05 was considered significant difference.

**RESULTS**

**In Vivo Study**

Effect of inhalation of epinephrine aerosol on rat vagal pulmonary afferents. A total of 25 pulmonary C-fiber afferents were studied in 17 anesthetized, open-chest rats. The distribution of locations of these receptors was as follows: 5 in the upper lobe, 11 in the middle lobe, 8 in the lower lobe, and 1 in the accessory lobe of the right lung.

Inhalation of epinephrine aerosol at both low and high concentrations markedly enhanced the pulmonary C-fiber response to lung inflation (Figs. 1 and 2). This potentiating effect sustained a substantially longer duration after the high concentration of epinephrine was administered.

In comparison, a significant potentiating effect on C-fiber response to capsaicin was only found after the challenge with high concentration of epinephrine (Fig. 2, bottom). In addition,
inhalation of epinephrine aerosol increased the baseline activity of the pulmonary C-fibers in a dose-dependent manner (Fig. 2, top). This stimulatory effect of epinephrine had a rapid (10 min) onset and returned slowly to control 10 and 60 min after deliveries of the low and high concentrations of epinephrine, respectively.

Inhalation of low concentration of epinephrine aerosol did not cause any significant change in either ABP or heart rate, whereas high concentration of epinephrine induced an intense hypertension (ΔABP = 37.5 mmHg; n = 8) during aerosol delivery, but this hypertensive response was short lasting (compared with the effect of epinephrine on C-fiber sensitivity) and completely returned to control in <10 min after termination of aerosol delivery.

In contrast, we did not find any detectable effect of epinephrine aerosol on the activity of either SARs or RARs. For example, the average FA of SARs in response to lung inflation did not change significantly after termination of aerosolized epinephrine treatment (pre-aerosol control: 91.0 ± 29.1 imp/s; 2 min after epinephrine: 127.3 ± 34.1 imp/s; P = 0.15; n = 8). Furthermore, a similar pattern of response was found after the delivery of vehicle (5 mg/ml ascorbic acid in saline) in the same group of receptors (pre-aerosol control: 95.4 ± 32.3 imp/s; 2 min after saline: 102.3 ± 29.7 imp/s; P = 0.55; n = 8). Similarly, epinephrine did not cause any detectable effect on the average FA response of RARs to lung inflation (n = 5).

**In Vitro Study**

Epinephrine potentiated the chemical stimulation-evoked Ca\(^{2+}\) transients in rat vagal sensory neurons. Consistent with what we have demonstrated in a previous study (12), application of capsaicin (50 nM, 15 s) evoked a reversible Ca\(^{2+}\) transient in small- and medium-size (diameter <35 μm) nodose and jugular ganglion neurons. As shown in Fig. 3A, epinephrine pretreatment (3 μM, 5 min) alone did not have any significant effect on the basal [Ca\(^{2+}\)] but dramatically increased the peak Ca\(^{2+}\) transient evoked by capsaicin. The group data shown in Fig. 3C illustrated that the capsaicin-induced Ca\(^{2+}\) transient was significantly increased after the epinephrine pretreatment by 106% (control: 55.8 ± 12.6 nM; after epinephrine: 115.1 ± 24.7 nM; washout: 75.2 ± 15.7; P < 0.05; n = 11). Pretreatment with the vehicle of epinephrine had no detectable effect on the capsaicin-induced Ca\(^{2+}\) transient (P = 0.81; n = 8) (Fig. 3, B and C).

The potentiating effect of epinephrine was not limited only to respond to capsaicin, the Ca\(^{2+}\) transient evoked by KCl (15 mM, 15 s) was also significantly potentiated after pretreatment with epinephrine (3 μM, 5 min) (Fig. 4). The KCl-evoked Ca\(^{2+}\) transient was elevated after epinephrine pretreatment in both capsaicin-sensitive (control: 83.3 ± 9.7 nM; after epinephrine: 130.5 ± 20.1 nM; washout: 96.3 ± 18.2; P < 0.05; n = 6) and capsaicin-insensitive neurons (control: 86.4 ± 8.3 nM; after epinephrine: 145.2 ± 23.4 nM; washout: 96.3 ± 18.2; P < 0.05; n = 6).
nM; after epinephrine: 126.5 ± 16.5 nM; washout: 92.6 ± 14.9; P < 0.05; n = 9) (Fig. 4D). In addition, pretreatment with the same dose of epinephrine potentiated the ATP (0.3 μM, 15 s)-evoked Ca²⁺ transient from a control level of 102.1 ± 11.6 nM to 143.5 ± 9.8 nM after the treatment (P < 0.05; n = 8). We should point out that the responses to these chemical stimuli and also the potentiating effect of epinephrine varied considerably among different cells (e.g., Figs. 3 and 4).

The potentiating effect of epinephrine on pulmonary vagal sensory neurons identified by Dil labeling was investigated in separate groups of neurons. Our results showed no significant difference in the capsaicin (50 nM, 15 s) and KCl (15 mM, 15 s)-evoked Ca²⁺ transients between labeled neurons and nonlabeled ones matched in size from the same cultures, either during control or after epinephrine pretreatment (Fig. 5).

Selective activation of α₁-, α₂-, β₁-, or β₂-adrenoceptor did not alter the sensitivity of rat vagal sensory neurons. Because epinephrine has affinity for both α- and β-adrenoceptors, our first approach was to determine whether activation of either or both of these major types of adrenoceptor could mimic the potentiating effect of epinephrine. As shown in Fig. 6, pretreatment with neither phenylephrine (5 μM, 5 min; P = 0.74; n = 12), an α₁-adrenoceptor agonist, nor guanabenz (5 μM, 5 min; P = 0.35; n = 11), an α₂-adrenoceptor agonist, altered the vagal sensory neuron responses to capsaicin (50 nM, 15 s) (Fig. 5, A and B). Similarly, pretreatment with either β₁-adrenoceptor agonist dobutamine (5 μM, 5 min; P = 0.23; n = 10) or β₂-adrenoceptor agonist salbutamol (5 μM, 5 min; P = 0.16; n = 10) had no significant effect on capsaicin (50 nM, 15 s)-evoked Ca²⁺ transient (Fig. 6, C and D).

The effect of epinephrine was mediated through β₂-adrenoceptor in rat vagal sensory neurons. As illustrated in Fig. 7A, pretreatment with BRL 37344 (5 μM, 5 min), a selective β₂-adrenoceptor agonist, produced a similar potentiating effect as epinephrine. The group data showed that capsaicin (50 nM, 15 s)-evoked Ca²⁺ transient was significantly increased from the control level of 97.5 ± 13.0 nM to 204.4 ± 39.9 nM after BRL 37344 (P < 0.05; n = 17) (Fig. 7B). Pretreatment with another selective β₂-adrenoceptor agonist ICI 215,001 (5 μM, 5 min) also significantly potentiated the capsaicin-evoked Ca²⁺ transient but to a lesser extent (control: 86.3 ± 15.6 nM; after ICI 215,001: 127.7 ± 19.9 nM; P < 0.05; n = 13) (Fig. 7, C and D). Furthermore, pretreatment with SR59230A (5 μM, 10 min), a selective β₂-adrenoceptor antagonist, completely abolished the potentiating effect of epinephrine on the Ca²⁺ transients evoked by both capsaicin (P = 0.23; n = 14) and KCl (P = 0.24; n = 15) (Fig. 8), whereas pretreatment with SR 59230A alone did not affect the sensory neuron responses to these two chemical stimulants (P = 0.88; n = 12).

Adenylate cyclase inhibitor abolished the effect of epinephrine in rat vagal sensory neurons. To investigate whether the activation of adenylate cyclase and subsequent increase of intracellular cAMP is critical to the potentiating effect of epinephrine, we tested the effect of SQ22536, a membrane-permeable adenylyl cyclase inhibitor. As shown in Fig. 9, pretreatment with SQ22536 (100 μM, 15 min) completely prevented the potentiating effect of epinephrine on capsaicin-evoked Ca²⁺ transient (P = 0.32; n = 8) (Fig. 9, A and B). In addition, pretreatment with SQ22536 also prevented the effect of epinephrine on the Ca²⁺ transient induced by KCl (P = 0.44; n = 16) (Fig. 9, C and D).

Inhibition of PKA prevented the epinephrine-induced potentiation in rat vagal sensory neurons. To determine whether the potentiating effect of epinephrine was due to the cAMP activation of PKA, we examined the effect of H89, a membrane-permeable PKA inhibitor, on the epinephrine-mediated enhancement of chemical stimulation-induced Ca²⁺ transient. Pretreatment with H89 (3 μM, 15 min) completely abolished the potentiating effect of epinephrine on the Ca²⁺ transients evoked by both capsaicin (P = 0.87; n = 10) and KCl (P =
DISCUSSION

β-Adrenoceptors are among the most extensively characterized members of the G-protein-coupled receptors. Three different subtypes of β-adrenoceptor (β₁, β₂, and β₃) have been characterized on the basis of biochemical, functional, and receptor-cloning studies. Although the fourth adrenoceptor (β₄) has been reported in heart muscle (17), it is now recognized that this newly proposed β-adrenoceptor most likely represents only a particular state of the β₁-adrenoceptor (25).

Functional assignment of β-adrenoceptor subtypes suggests that β₁-adrenoceptor is the predominant subtype regulating heart rate and contractility, although all three β-adrenoceptors appear to be involved in the cardiac stimulation (17). β₂-Adrenoceptor is the predominant subtype mediating the vascular smooth muscle-relaxant properties (32). β₃-Adrenoceptor was initially identified in adipose tissue, and its presence has been recently demonstrated in a variety of tissues including blood vessel (32), gastrointestinal tract (9), urinary bladder smooth muscle (43), and retinal endothelial cell (37). In the present study, our results have demonstrated that, in anesthetized, open-chest, and artificially ventilated rats, inhalation of epinephrine aerosol substantially enhanced pulmonary C-fiber responses to lung inflation and capsaicin injection (Figs. 1 and 2). Our data have also shown that pretreatment with epinephrine significantly potentiated chemical stimulation-evoked Ca²⁺ transient in isolated rat vagal sensory neurons including those specifically innervating the lungs (Figs. 3–5). In addition, the potentiating effect of epinephrine was mimicked by both BRL 37344 and ICI 215,001, two selective β₃-adrenoceptor agonists (Fig. 7), and abolished by SR 59230A, a selective β₃-adrenoceptor antagonist (Fig. 8), whereas pretreatment with none of the α₁-, α₂-, β₁-, and β₂-adrenoceptor agonists had any significant effect on capsaicin-evoked Ca²⁺ transient (Fig. 6). Taken together, these results strongly suggest that activation of β₃-adrenoceptor mediates the sensitizing actions of epinephrine.
rine on these vagal sensory neurons, although additional evidence obtained from immunohistochemistry and/or molecular cloning will be required to further confirm the expression of this specific β-adrenoceptor in these neurons.

All three β-adrenoceptor subtypes are known to be coupled to the Gs-protein, leading to the activation of adenylate cyclase and accumulation of the second messenger cAMP, which has been demonstrated both in native tissues and in reconstitution systems (13, 38). It has been proposed that epinephrine, acting on nociceptors, produces mechanical hyperalgesia through three different signaling pathways involving 1) cAMP-dependent PKA; 2) epsilon isozyme of PKC; and 3) extracellular signal-regulated kinases 1 and 2 (1, 20). It has been demonstrated that the latter two pathways may also be initiated by the accumulation of cAMP (8, 35). However, recent pharmacological and electrophysiological studies have reported the presence of β-adrenoceptor-mediated cAMP-independent signaling pathways (38); for example, the β3-adrenoceptor-induced relaxation of gastrointestinal smooth muscle has been proposed to be mediated through the activation of a delayed rectified K+ channel (15). In the present study, our results suggest that intracellular cAMP-PKA transduction cascade is substantially

Fig. 6. Effects of α₁-, α₂-, β₁-, and β₂-adrenoceptor agonists on the capsaicin-evoked Ca²⁺ transient in rat vagal sensory neurons. A–D: effects of pretreatments with phenylephrine (α₁-adrenoceptor agonist; 5 μM, 5 min; n = 12), guanabenz (α₂-adrenoceptor agonist; 5 μM, 5 min; n = 11), dobutamine (β₁-adrenoceptor agonist; 5 μM, 5 min; n = 10), and salbutamol (β₂-adrenoceptor agonist; 5 μM, 5 min; n = 10), respectively, on the Ca²⁺ transient evoked by Cap (50 nM, 15 s). Data are means ± SE.

Fig. 7. Effects of β₃-adrenoceptor agonists on the capsaicin-evoked Ca²⁺ transient in rat vagal sensory neurons. A and C: experimental records illustrating the effects of pretreatments with BRL 37344 (β₃-adrenoceptor agonist; 5 μM, 5 min) and ICI 215,001 (β₃-adrenoceptor agonist; 5 μM, 5 min), respectively, on the Ca²⁺ transient evoked by Cap (50 nM, 15 s). B and D: group data showing the effects of pretreatments with BRL 37344 (n = 17) and ICI 215,001 (n = 13), respectively, on the capsaicin-evoked Ca²⁺ transient. Data are means ± SE. *Significantly different from the control response (P < 0.05).
involved in, if not solely responsible for, the sensitizing effect of epinephrine in rat vagal sensory neurons, since pretreatment with either adenylyl cyclase inhibitor SQ 22536 (Fig. 9) or PKA inhibitor H89 (Fig. 10) completely prevented the potentiating effect of epinephrine in these neurons.

We have recently demonstrated that capsaicin evoked a Ca\(^{2+}\) transient in small- to medium-size rat vagal sensory neurons; the response was mediated through transient receptor potential vanilloid receptor type 1 (TRPV1) and was dependent on the extracellular Ca\(^{2+}\) (12). TRPV1, a member of the transient receptor potential ion channel superfamily (27), is a polymodal nonspecific cation channel expressed predominantly in unmyelinated (C) fibers and is activated not only by capsaicin but also by noxious heat, protons, anandamide, and lipoygenase metabolites (4, 16). Therefore, TRPV1 may act as a thermal and chemical transducer and contribute to neurogenic inflammation (4). It has been demonstrated that cAMP-dependent PKA activation can enhance capsaicin-induced inward current and Ca\(^{2+}\) transient in dorsal root ganglion neurons (36) as well as in vagal sensory neurons (12, 22). Indeed,
recent studies have successfully delineated several candidate
PKA-phosphorylation sites in TRPV1, through which PKA
may mediate the sensitization of this channel as well as prevent
its desensitization (2).

ATP, another chemical stimulant used in this study, is
known to activate the ligand-gated P2X3 purinoceptor that is
coupled to nonselective cation channel and widely expressed in
both the central and peripheral nervous systems (31). PKA-
dependent sensitization of TRPV1 and P2X3 may increase the
permeability of these channels to cations such as Ca2+ and Na+,
which can lead to cell membrane depolarization and
subsequent activation of voltage-dependent Ca2+ channels
(VDCCs). On the other hand, VDCCs are also the potential
phosphorylation targets of PKA activation, which may increase
the channel availability (19) or modulate the channel properties
(10). This assumption is supported by our observation that
epinephrine also potentiates the KCl-evoked Ca2+ transient
(Fig. 4), a response resulting from the cell membrane depolar-
ization and subsequent activation of VDCCs. We cannot com-
pare the extent of the potentiation of epinephrine on these three
chemical stimulants (capsaicin, ATP, and KCl) because a full
range of the dose responses of these activators was not estab-
lished in this study. Our in vitro study was carried out in
cultured neurons, and the responses were recorded from the
neuronal soma. Hence, we could not determine the specific
types of afferent terminals of these individual isolated neurons,
although we have purposely selected the small- to medium-size
neurons for this study. In fact, in the experiments testing the
responses to ATP and KCl, we did not exclude capsaicin-
insensitive neurons because our previous studies indicated that
~40% of small- to medium-size (diameter of <35 µm) nodose
and jugular ganglion neurons prepared under similar conditions
do not exhibit any sensitivity to capsaicin (6, 42). More
importantly, our in vivo single-fiber recording data clearly
indicate that the sensitizing effect of epinephrine is found
exclusively in C-fiber afferents and not in SARs or RARs in the
rat lungs. In addition, activation of TRPV1, P2X3, or VDCCs
may initiate Ca2+ release from intracellular stores via a process
of Ca2+-induced Ca2+ release (40). Indeed, previous studies
have provided direct evidence that Ca2+-induced Ca2+ release
can be triggered solely by Ca2+ influx in various sensory
neurons including dorsal root (36) and nodose (7) ganglion
neurons. On the basis of our results, we cannot rule out the
possibility that increased Ca2+-induced Ca2+ release may have
also contributed to the enhanced chemical stimulation-evoked
Ca2+ transients after the epinephrine pretreatment.

The present study was carried out using two different ex-
perimental approaches. We used the in vivo single-fiber rec-
dording to determine the effect of epinephrine in intact animals
and the in vitro Ca2+ imaging to identify the subtypes of
adrenoceptor mediating this effect in isolated vagal sensory
neurons. Whether there are differences in the receptor concen-
trations and the sensitivities of these receptors between the
sensory terminal and the soma of these neurons remains to be
determined. Nevertheless, our study has shown that inhalation
of epinephrine aerosol markedly enhanced pulmonary C-fiber
sensitivity in anesthetized rats and that epinephrine pretreat-
ment significantly potentiated chemical stimulation-evoked
Ca2+ transient in isolated rat vagal sensory neurons; these
results are in general agreement with the previous findings
from other investigators (1, 5, 21, 28). Under the conditions of
acute stress, the systemic plasma concentration of epinephrine
increases dramatically in various species, including humans
(41). Intradermal administration of epinephrine has been
shown to produce mechanical and thermal hyperalgesia (5, 21),
which may be explained by a direct sensitizing effect of
epinephrine on primary nociceptive afferents (1, 21). It has
been recently reported that neural discharge in vagal afferent
fibers is significantly increased by elevations of peripheral
concentrations of epinephrine in rats (28).

The primary afferents innervating various visceral organs
are mainly conducted through vagus nerves. It is known that

![Fig. 10. Effect of H89 on the potentiation of capsaicin- and KCl-evoked Ca2+ transients by Epi in rat vagal sensory neurons. A and B:
experimental records illustrating the effects of pretreatments with H89 (3 µM, 15 min), a membrane permeable PKA inhibitor, and its
vehicle, respectively, on the potentiation of Cap (50 nM, 15 s)-evoked Ca2+ transient by Epi (3 µM, 5 min). C: group data showing the
effects of H89 (n = 10) and its vehicle (n = 8) on the potentiating effect of Epi on the Ca2+ transient evoked by Cap (50 nM, 15 s).
D: group data showing the effects of H89 (n = 9) and its vehicle (n = 9) on the potentiating effect of Epi on the Ca2+ transient
evoked by KCl (15 mM, 15 s). Data are means ± SE. *Significantly different from the corresponding response to Cap or KCl alone (P < 0.05).
†Significant difference between the corresponding data obtained from pretreatments with H89 and its vehicle (P < 0.05).](http://dx.doi.org/10.2307/124781)
the chemosensitive vagal afferent endings can be stimulated by various endogenous substances, such as H\(^+\), adenosine, and serotonin (24, 29), and by various inhaled irritants such as cigarette smoke, SO\(_2\), ozone, and also lung expansion (24). Under normal physiological conditions, these stimuli (endogenous chemicals, inhaled irritants, and lung expansion) may not generate a significant stimulatory effect on the vagal afferent terminals. However, when the excitability of these afferents is upregulated by epinephrine, as shown in the present study, their stimulation thresholds will be lowered (e.g., Fig. 1B), which may then lead to afferent activation. In addition, some of these endogenous activators (e.g., H\(^+\)) are known to be secreted at high concentrations under acute stressful conditions (e.g., strenuous exercise), during which the circulating level of epinephrine is also expected to increase substantially (30). Therefore, the consequent augmented visceral/visceroresponsive reflexes may, presumably, contribute to the adaptive responses of these visceral organs to the acute stresses and play a role in maintenance of homeostasis under these conditions.

In summary, our results show that inhalation of epinephrine aerosol induced a pronounced increase in the sensitivity of pulmonary C-fibers to lung inflation and right atrial injection of capsaicin. Furthermore, pretreatment with epinephrine markedly and consistently potentiated the chemical-stimulation-evoked Ca\(^{2+}\) transients in isolated rat vagal sensory neurons. The effect of epinephrine was mimicked by either BRL 37344 or ICI 215,001, two specific β\(_1\)-adrenoceptor agonists, and blocked by SR 59230A, a selective β\(_1\)-adrenoceptor antagonist. In contrast, pretreatment with none of α\(_1\)-, α\(_2\)-, β\(_1\)-, or β\(_2\)-adrenoceptor agonists had any significant effect. Furthermore, pretreatment with either adenylyl cyclase inhibitor SQ 22536 or PKA inhibitor H89 completely blocked the potentiating effect of epinephrine. Taken together, these results suggest that epinephrine upregulates the sensitivity of rat vagal chemosensitive neurons; this sensitizing effect is probably mediated through the β\(_3\)-adrenoceptor and the subsequent activation of intracellular cAMP-PKA transduction cascade.

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