Selective stimulation of jugular ganglion afferent neurons in solenia pig airways by hypertonic saline

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Pedersen, Karen E., Sonya N. Meeker, Margerita M. Riccio, and Bradley J. Undem. Selective stimulation of jugular ganglion afferent neurons in guinea pig airways by hypertonic saline. J. Appl. Physiol. 84(2): 499–506, 1998.—We evaluated the ability of hyperosmolar stimuli to activate afferent nerves in the guinea pig trachea and main bronchi and investigated the neural pathways involved. By using electrophysiological techniques, studies in vitro examined the effect of hyperosmolar solutions of sodium chloride (hypertonic saline) on guinea pig airway afferent nerve endings arising from either vagal nodose or jugular ganglia. The data reveal a differential sensitivity of airway afferent neurons to activation with hypertonic saline. Afferent fibers (both Aδ and C fibers) with cell bodies located in jugular ganglia were much more sensitive to stimulation with hypertonic saline, compared with afferent neurons with cell bodies located in nodose ganglia. Additional studies in vivo demonstrated that inhalation of aerosols of hypertonic saline induced plasma extravasation in guinea pig trachea that was mediated via tachykinin NK1 receptors. Identification of a differential sensitivity of guinea pig airway afferent nerves to hypertonic saline leads to the speculation that airway responses to hyperosmolar stimuli may result from activation of afferent neurons originating predominantly from the jugular ganglion.

osmolar concentration; vagus nerve; tachykinin; neuropeptides; asthma

AFFERENT NERVES innervating the respiratory tract are an integral component of the neural network that serves to regulate airway function. In the airways, activation of afferent nerve endings leads to the transmission of action potentials to the central nervous system where they can evoke reflex actions including cough, sneezing, and changes in the rate and depth of breathing (2, 28). In addition, activation of airway afferent neurons may also influence airway function locally via antidiromic release of neuropeptides from sensory nerve terminals within the airway wall. Among such neuropeptides are the tachykinin family, whose members include substance P (SP) and neurokinin A, which have been shown to possess numerous effects on airway tissues, including effects on airway smooth muscle as well as potent proinflammatory actions (see Ref. 28).

A number of different stimuli are known to activate airway afferent nerves. These include changes in mechanical force, changes in pH, various chemicals (e.g., CO2 and autacoids), and changes in osmolarity (2, 28). That airway afferent fibers are sensitive to changes in the osmolarity of their environment may have important consequences under conditions whereby the normal composition of the fluid layer lining the airways is altered. Indeed, it has been proposed that evaporative water loss leading to an increase in osmolarity of the fluid lining the surface of the airway mucosa may represent a mechanism underlying exercise-induced asthma (9, 23). However, while both hyper- and hyposmolar solutions have been shown to stimulate airway afferent neurons (7, 20, 21), relatively little is known regarding the specific neural pathways involved.

In the trachea and main bronchi, the predominant afferent supply appears to be vagal in origin in that the somata of afferent fibers innervating this region are located almost exclusively in the nodose and jugular ganglia (3, 14, 26). Recently, findings from several studies have indicated that afferent neurons arising from vagal nodose and jugular ganglia may represent neurochemically and functionally distinct phenotypes. Studies in the isolated guinea pig trachea-bronchus show that there are marked differences in the responses of airway vagal afferent fibers to chemical and mechanical stimuli (21). In addition, anatomic investigations have revealed that, in the guinea pig, SP-, neurokinin A-, dynorphin-, and calcitonin gene-related peptide-immunoreactive neurons project to the trachea from jugular but not nodose ganglia (14, 21) and that, in the rat, calcitonin gene-related peptide-containing neurons innervating the trachea have their cell bodies located in jugular ganglia but rarely in nodose ganglia (26). Thus, whereas vagal afferent airway nerves are derived from both the nodose and jugular vagal ganglia, the cell bodies of tachykinin-containing vagal airway afferent neurons appear to be present mainly in the jugular ganglion.

In the present study, we were interested in investigating the effects of hyperosmolar stimuli on afferent nerve activity in guinea pig airways. To examine the neural pathways involved, electrophysiological investigations were performed in vitro, in which afferent nerve endings arising from either jugular or nodose vagal ganglia were exposed to hyperosmolar solutions of sodium chloride (NaCl). Additional studies were also conducted in vivo, in which guinea pig airways were challenged with aerosols of hypertonic saline, to investigate physiological consequences of airway exposure to hypertonic saline in this species. In these latter investigations, increased microvascular permeability was used as a physiological marker of sensory nerve activation, as plasma extravasation is a well-characterized component of neurogenic inflammation in the guinea pig respiratory tract (5, 16, 17). Findings from the present study show hypertonic saline to activate guinea pig airway afferent nerves in vitro and in vivo and demon-
strate that there is a ganglionic dependence of airway afferent vagal neurons to activation with this stimulus.

METHODS

Electrophysiological studies. Electrophysiological investigations were conducted by using an in vitro guinea pig tracheal and bronchial preparation with intact afferent vagal pathways, according to a method previously described (21). Male Dunkin-Hartley guinea pigs weighing 100–200 g (Harlan Sprague-Dawley, Indianapolis, IN) were killed by CO2 asphyxiation and exsanguinated. The airways and associated right-side vagal innervation (vagus, superior laryngeal, and recurrent laryngeal nerves) with attached nodose and jugular ganglia were removed and placed in oxygenated Krebs-bicarbonate buffer of the following composition (mM): 118 NaCl, 5.4 KCl, 1.0 NaH2PO4, 1.2 MgSO4, 1.9 CaCl2, 25 NaHCO3, and 11.1 dextrose. The larynx, airways, neural pathways, and ganglia were carefully cleared of surrounding connective tissue, and the larynx, trachea, and main bronchi were opened along their ventral surface by a midline incision. The preparation was then pinned out flat to Sylgard in one compartment of a two-compartment Perspex chamber with the airway luminal surface upward. The right nodose and jugular ganglia along with attached vagus and superior laryngeal nerves were gently pulled through a small hole into the adjacent compartment where single-nerve fiber activity was measured. Both chambers were perfused separately with oxygenated, warmed (37°C) Krebs-bicarbonate buffer at a flow rate of 6–8 ml/min. Recording of action potentials. Extracellular recording was performed by positioning a fine aluminum glass microelectrode filled with 3 M NaCl solution (electrode resistance, ~2 MΩ) near neuronal cell bodies in either the nodose or jugular ganglion. The recorded signal was amplified and the resultant activity displayed on an oscilloscope (TDS 320; Tektronix, Wilsonville, OR). Data were stored on magnetic tape by using a digital audiotape recorder (DTC 59ES; sampling frequency, 22 kHz; Dagan, Minneapolis, MN), and the recorded action-potential discharge was analyzed off-line by using a customized spike discrimination and counting software program (D. M. MacGlashan, PHOCUS, Baltimore, MD). The number of action potentials recorded was then imported into a spreadsheet package (Microsoft Excel, version 4.0) for further data handling and analysis.

Determination of single-fiber activity and detection of afferent nerve terminals. Single-fiber activity in the airways was discriminated by placing an electrical stimulating electrode on the recurrent laryngeal nerve while the recording electrode was carefully manipulated into and out of different locations in the nodose or jugular ganglion until single-unit activity was detected. When electrically evoked action potentials were seen, the stimulus was switched off, and the trachea and bronchi were gently probed by using a fine blunt plastic rod (OD 2 mm) to locate the mechanically sensitive receptive field. Identification of the mechanically sensitive receptive field was made when touching of a specific area of the airway luminal surface elicited a burst of action potentials. In some instances, we failed to find the receptive field when using mechanical search techniques. These mechanically insensitive neurons were not studied further.

Conduction velocities. Conduction velocities of afferent fibers were determined by electrically stimulating the receptive field and monitoring the time elapsed between appearance of the shock artifact and action potential. This was then divided by the distance traveled along the nerve trunk to the recording microelectrode to estimate the conduction velocity. Fibers were classified as C fibers if they conducted action potentials at <1.3 m/s and as Aδ fibers if they conducted action potentials >2.0 m/s (29). Fibers propagating action potentials between 1.3 and 2.0 m/s could fall into either category and were thus excluded from the analyses.

Mechanical stimulation. Mechanical thresholds were determined for each nerve ending by using von Frey filaments (Stoelting, Wood Dale, IL) calibrated to give fixed amounts of force ranging from 0.078 to 2738 mN. Beginning with the lowest force, von Frey filament nerve endings were gently probed with filaments of increasing force until a threshold mechanical sensitivity was determined. This was achieved when touching of the receptive field evoked a burst of action potentials. Confirmation of threshold sensitivity was established by probing the nerve ending with the subthreshold filament.

Hyperosmolar stimuli. After characterization of mechanical sensitivity, the effects of hyperosmolar solutions of NaCl on airway afferent nerve activity were determined. Hyperosmolar solutions of NaCl were prepared by addition of an appropriate amount of NaCl to 10 ml of the Krebs-bicarbonate buffer to give solutions (NaCl-Krebs) with final concentrations of NaCl ranging from 0.9 to 7% (wt/vol). Following preparation, solution temperature was maintained at 37°C by using a heated water bath. The effects of hypertonic saline on the activity of jugular ganglion or nodose ganglion fibers were established by adding a 250-μl volume of NaCl-Krebs solution directly over the receptive field. Beginning with the lowest concentration of NaCl (0.9%), the 250-μl bolus of solution was added by using a 1-ml transfer pipette in ~3 s. With individual units, if no response was observed, the next highest concentration of solution was applied after a period of 2 min. When addition of the NaCl-Krebs solution elicited action-potential formation, the next highest concentration was added 2 min after cessation of firing. It should be noted that the receptive field was being simultaneously superfused with the Krebs-bicarbonate buffer, and thus the actual saline challenge may have been less than the concentration of NaCl added. However, all tissues were treated in an identical fashion allowing for comparison of responses.

In addition to testing the effect of hypertonic saline, we also examined the effect of a second hyperosmolar stimulus, mannitol, on the activity of guinea pig jugular ganglion fibers. Mannitol, a compound devoid of sodium and chloride ions, was prepared as a 0.9-M solution by dissolving the compound in distilled water and warming the solution to 37°C. Determination of solution osmolarity with the use of an osmometer (Wescor 5500 vapor pressure osmometer; Wescor, Logan, UT) revealed that at a concentration of 0.9 M the osmolarity of the mannitol solution was 1,233 ± 116 mosmol (n = 3), which was comparable to that of the 4% NaCl-Krebs solution (osmolarity = 1,264 ± 2 mosmol; n = 3).

The effect of mannitol on the response of mechanically sensitive airway fibers was established by adding a 250-μl bolus of mannitol solution directly over the receptive field in a manner identical to that for hypertonic saline. In experiments in which the effect of mannitol was investigated, the mannitol solution was added some 2 min after cessation of firing of the afferent neuron in response to challenge with 4% NaCl-Krebs solution.

Determination of superfusate osmolarity. As the receptive field was being simultaneously superfused with Krebs-bicarbonate buffer, it was desirable to obtain an estimate of the change in osmolarity of the superfusate over the airway tissue preparation after addition of hypertonic saline. Experiments were thus conducted in which samples of the airway chamber superfuse were collected at various time points after addition of the NaCl-Krebs solution, and the osmolarity
of these samples was measured. In these experiments, a 50-µl sample of superfusate was taken immediately after application of the NaCl-Krebs solution and then at intervals extending out to 5 min. Samples were collected from a region as close to the receptive field as possible, and their osmolarity was determined with the use of an osmometer.

Plasma extravasation studies. Male guinea pigs (Dunkin-Hartley; Harlan Sprague-Dawley) weighing 200–300 g were used in all plasma extravasation studies. Guinea pigs were anesthetized with urethane (2 g/kg ip), and the right jugular vein was cannulated for the intravenous administration of agents. The larynx and upper trachea were exposed, the trachea immediately below the larynx was incised, and a cannula was inserted 5 mm into the airway. The animal was then connected to a small-animal respirator (Harvard rodent ventilator model 683; Harvard Apparatus, South Natick, MA) and ventilated artificially with humidified air at a rate of 60 breaths/min and a tidal volume of 8 ml/kg. Guinea pigs received Evans blue dye (30 mg/kg iv), and 1 min later an aerosol of NaCl solution (0.9–7%, wt/vol, nebulizer concentration) was administered. In in vivo studies, saline solutions were prepared by addition of an appropriate amount of NaCl to 10 ml of sterile distilled water to give solutions with final concentrations of NaCl ranging from 0.9 to 7%. Aerosols were generated by using an ultrasonic nebulizer (Pulmo-Sonic model 25; DeVibiss, Somerset, PA) and delivered into the airways via the tracheal cannula for 2 min during ventilation with the respirator. Control animals received no aerosol of NaCl solution.

Ten minutes after the end of the aerosol administration, the chest was opened and a cannula inserted into the ascending aorta through an incision in the left ventricle. The circulation was then perfused with 250 ml of 0.9% saline to expel intravascular dye. The lungs and trachea were removed and the trachea and main bronchi dissected free and cleared of connective tissue. Airway tissues were gently blotted on filter paper to remove excess moisture and then weighed. The amount of Evans blue dye in airway tissues was determined by extraction of dye in formamide for 24 h at 37°C. Extravasation of Evans blue dye in airway tissues was quantified by measuring the optical density of the formamide extracts at 620 nm, a wavelength in a spectrophotometer (Spectronic 1201; Milton Roy, Rochester, NY). The amount of Evans blue dye in airway tissues was interpolated from a standard curve of Evans blue concentrations (0.5–10 µg/ml) and expressed as nanograms dye per milligram wet weight tissue.

To investigate potential involvement of tachykinin-containing sensory nerves in the airway microvascular leakage induced by hypertonic saline aerosol, the effect of the selective neurokinin 1 (NK1)-receptor antagonist 1-[2-(3-(4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl)[ethyl]-4-phenyl-1-azonia-bicyclo[2.2.2]octane chloride (SR-140333) (13) on the plasma extravasation response was examined. In these experiments, SR-140333 (1 µmol/kg) or vehicle (1% dimethyl sulfoxide in 0.9% saline, 2 ml/kg) was administered intravenously 15 min before the start of the NaCl aerosol, and the effect of hypertonic saline on airway microvascular permeability was assessed as described above.

Drugs and solutions. Substance P, Evans blue dye, and α-mannitol were purchased from Sigma Chemical (St. Louis, MO); SR-140333 was a generous gift from Zeneca Pharmaceuticals Group (Willmington, DE). SR-140333 was prepared as a stock solution (5 × 10⁻² M) in dimethyl sulfoxide and diluted to the final concentration (0.5 µmol/ml) in 0.9% saline solution. All other drugs used in in vivo studies were dissolved in 0.9% saline, and solutions of hypertonic saline, mannitol, and of all drugs were prepared fresh on the day of experimentation.

Data analysis. Mechanical thresholds are presented as the threshold von Frey filament to elicit a response. Mechanical thresholds were not normally distributed, and thus these data were log₁₀ transformed before statistical analysis. Mean values for mechanical thresholds are presented as geometric means together with the upper and lower limits of the SE. All other data are presented as arithmetic means ± SE. Responses of neurons to hyperosmotic stimuli are presented graphically as the total number of action potentials elicited in response to the application of the NaCl or mannitol solution. Electrophysiological and microvascular leakage data were compared by using a one-way analysis of variance followed by Student’s nonpaired t-test. For both electrophysiological and in vivo studies, differences having a P value < 0.05 were considered significant.

RESULTS

Electrophysiological investigations: general characteristics. A total of 37 afferent fibers supplying the trachea and main stem bronchi from 31 guinea pigs was investigated. Of these, the cell bodies of 23 fibers were located in the jugular ganglion, with the cell bodies of 14 fibers situated in the nodose ganglion. The majority of afferent fibers showed virtually no spontaneous activity, and the small percentage of fibers that were found to be spontaneously active (~5%) were not included.

In the present study, 10/23 of the jugular ganglion neurons investigated projected fibers that conducted action potentials in the Aδ range, whereas 13/23 jugular neurons conducted action potentials in the C-fiber range. For the nodose ganglion, 11/14 and 3/14 fibers conducted action potentials in the Aδ- and C-fiber ranges, respectively. The mean values and range of the conduction velocities for the vagal jugular and nodose ganglion fibers investigated are listed in Table 1. Only one fiber was found that conducted in the intermediate range (i.e., 1.3–2.0 m/s). This fiber, which arose from nodose somata, was excluded from the study. As our search paradigm included mechanical probing to locate the receptive field, all fibers studied were mechanically sensitive. The mechanical thresholds for activating jugular ganglion Aδ- and C fibers and nodose ganglion Aδ fibers are also listed in Table 1. The mechanical threshold of nodose ganglion Aδ fibers was found to be significantly lower compared with both jugular ganglion Aδ- (P < 0.001) and jugular ganglion C-fiber (P < 0.001) neurons. For nodose ganglion C fibers, the mechanical threshold of two nodose C fibers examined averaged 0.56 mN.

Responses to hyperosmolar stimuli. Application of increasing concentrations of NaCl-Krebs solution directly over the receptive field of mechanically sensitive fibers in the guinea pig trachea and main bronchi caused a concentration-related increase in action-potential discharge from neurons with cell bodies located in the jugular ganglion (Fig. 1) (n = 23). This response was observed for both jugular ganglion Aδ fibers (n = 10) and jugular ganglion C fibers (n = 13). In contrast, neurons with cell bodies located in the nodose...
ganglion were much less sensitive to the effects of hypertonic saline solutions (Fig. 1) \((n = 14)\). In the present study, 11 out of 14 nodose ganglion neurons conducted action potentials in the A\(\delta\) range (\(\sim 2.0\) m/s). For these fibers, appreciable action-potential discharge was only observed at the higher concentrations of NaCl solution examined (i.e., 6 and 7% NaCl-Krebs), whereas at lower concentrations nodose ganglion A\(\delta\) fibers were essentially unresponsive to this stimulus. Relatively few C fibers originating from the nodose ganglia have been found to project to the guinea pig trachea-bronchus (21). Only 3 out of 14 nodose fibers conducted action potentials in the C-fiber range (\(\sim 1.3\) m/s) (Table 1) and, of these, 2 fibers responded to the hyperosmolar solutions of NaCl in a manner more similar to that of the jugular ganglion C fibers while 1 neuron failed to respond to hypertonic saline except at a concentration of 6–7% NaCl-Krebs, thus resembling the nodose ganglion A\(\delta\) fibers.

The response of jugular ganglion neurons to challenge with hypertonic saline often displayed a characteristic pattern of action-potential discharge, whereby there was perceptible delay between application of the NaCl-Krebs solution and recording of action potentials. In many instances, the observed pattern of response also included a brief initial burst followed by a delay with a variable pattern of firing thereafter (see Fig. 1). For many jugular ganglion fibers (both A\(\delta\) and C fibers), action-potential discharge lasted for a protracted period of time (often >60 s). This was most notable after application of higher concentrations of NaCl-Krebs solution.

As detailed in Methods, challenge of guinea pig airway afferent fibers with hypertonic saline in vitro was achieved by applying 250 µl of a known concentration of NaCl-Krebs solution directly over the receptive field. As the tissue preparation was simultaneously being superfused with isotonic buffer, the osmolarity of the solution reaching the afferent nerve ending may have been less than the actual concentration of NaCl.

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<th>Nodose Ganglia</th>
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<td>A(\delta) fibers</td>
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<td>7.6 ± 0.8 m/s</td>
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<td>Range</td>
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<td>C fibers</td>
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<td>Mean</td>
<td>0.9 ± 0.2 m/s</td>
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<td>Range</td>
<td>0.7–1.2 m/s</td>
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Conduction velocities are presented as arithmetic mean and range of conduction velocities for each fiber type. Mechanical thresholds are presented as geometric mean together with upper and lower limits of SE. \(n\) Values represent no. of fibers studied for each data point.

![Fig. 1. A: effect of hypertonic saline on afferent vagal nerve activity in guinea pig trachea and main stem bronchus. Each point represents mean ± SE of total no. of action potentials discharged in response to application of a 250-µl bolus of 0.9–7% NaCl-Krebs solution (see text) directly over receptive field of mechanically sensitive jugular ganglion fibers (A\(\delta\) or C fibers, \(\bullet\); \(n = 23\)) or mechanically sensitive nodose ganglion fibers (A\(\delta\) or C fibers, \(\bullet\); \(n = 14\)). Insets represent typical responses of jugular ganglion A\(\delta\) neuron (a; conduction velocity = 4.8 m/s) and nodose ganglion A\(\delta\) neuron (b; conduction velocity = 2.4 m/s) to challenge with NaCl-Krebs solution. (Note there are 2 readily discriminated units in the trace showing response of nodose fiber to 7% saline. In these instances, only the action potentials discharged by the unit corresponding to receptive field were included in numerical analyses). B: neurogram of frequency of action-potential discharge over time by jugular ganglion A\(\delta\) neuron in a. Each point represents no. of action potentials recorded in consecutive 2-s increments illustrating time course of response of this fiber to 4% NaCl-Krebs solution (total no. of action potentials = 324, peak frequency of firing = 11 Hz).]
HYPERTONIC SALINE STIMULATES JUGULAR GANGLION NEURONS

Fig. 2. Effect of hypertonic saline on afferent nerve activity in mechanically sensitive jugular ganglion fibers projecting to guinea pig trachea and main stem bronchus. Each point represents mean ± SE of total no. of action potentials discharged in response to application of a 250-µl bolus of 0.9–7% NaCl-Krebs solution (see text) directly over receptive field of jugular ganglion Aδ fibers (n = 13) or C fibers (n = 10). * Response significantly different from that observed in jugular ganglion Aδ fibers, P < 0.05.

As the ability of hypertonic saline to stimulate airway afferent fibers could have resulted from either an increase in osmolarity per se or an increase in the concentration of permeant ions, we also evaluated the effect of an equiosmolar solution of mannitol (a compound devoid of sodium and chloride ions) on afferent activity in the guinea pig trachea-bronchus. Application of an equiosmolar solution of mannitol mimicked the effect of the 4% NaCl-Krebs solution with respect to the activation of jugular ganglion fibers (n = 3) (Fig. 3). The effect of mannitol cannot be attributed to the lack of chloride ions, as a 250-µl bolus of isotonic glucose solution (6%) had no effect on 11 of 13 jugular Aδ fibers or 6 of 8 jugular C fibers (data not shown).

Effect of hypertonic saline aerosol on airway microvascular permeability. Extravasation of Evans blue dye in the trachea and main bronchi of artificially ventilated guinea pigs challenged with isotonic saline (0.9% nebulizer concentration) was 10.9 ± 2.2 ng/mg wet wt tissue (n = 10) and 11.0 ± 2.5 ng/mg wet wt tissue (n = 10), respectively. These values were not significantly different from those obtained in control animals that received no aerosol where extravasation of Evans blue dye was 14.1 ± 2.9 ng/mg wet wt tissue (n = 3) in the trachea and 8.5 ± 1.7 ng/mg wet wt tissue (n = 3) in the main bronchi. Inhalation of aerosols of hypertonic saline of 4 and 7% NaCl (nebulizer concentration) for 2 min caused a concentration-dependent increase in the extravasation of Evans blue dye in the guinea pig trachea and main bronchi (Fig 4). In the trachea, the increase in airway microvascular permeability induced by inhalation of hypertonic saline aerosol was abolished by pretreatment of animals with the selective NK₁-receptor antagonist SR-140333 (1 µmol/kg) (Fig. 5).

To verify the in vivo selectivity of SR-140333, the effect of hypertonic saline aerosol on airway microvascular permeability was further analyzed in guinea pigs challenged with isotonic saline (0.9% nebulizer concentration) for 2 min caused a concentration-dependent increase in the extravasation of Evans blue dye in the guinea pig trachea and main bronchi (Fig 4). In the trachea, the increase in airway microvascular permeability induced by inhalation of hypertonic saline aerosol was abolished by pretreatment of animals with the selective NK₁-receptor antagonist SR-140333 (1 µmol/kg) (Fig. 5).

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of this antagonist against histamine- and SP-induced increased microvascular permeability in the guinea pig trachea was examined. At a concentration of 1 µmol/kg, SR-140333 abolished the increase in microvascular permeability induced by intravenous SP (1 µg/kg) but had no effect on histamine (100 µg/kg iv) -induced microvascular leakage (data not shown).

### DISCUSSION

Results of the present study show that challenge of guinea pig airways with hyperosmolar solutions of NaCl leads to the activation of airway afferent nerves both in vitro and in vivo. The electrophysiological data confirm previous findings that both Aδ fibers and C fibers innervating the isolated guinea pig trachea and main stem bronchus are excited by solutions of hyper tonic saline (7, 21). However, the findings presented expand on previously reported observations in that they demonstrate clear differences in the sensitivity of guinea pig airway afferent vagal neurons to activation by hypertonic saline and that this differential sensitivity appears to be a function of the ganglionic origin of the fiber. It has been previously shown by our laboratory (21) that afferent neurons projecting to the guinea pig trachea and main bronchi from vagal nodose and jugular ganglia show distinct differences in sensitivity to activation with mechanical stimuli and in response to the chemical agent capsaicin. Data from the present study provide further evidence of an interganglionic segregation of functionally distinct types of afferent neurons projecting to the guinea pig central airways. We speculate that this distinction in physiological function may be attributed to the distinct embryological origin of vagal afferent neurons in the nodose and jugular ganglia (31). In any event, it is likely that the origin of the vagal afferent fibers innervating the airways is a key determinant in their biological function.

Several distinctions were notable in comparison of the responses of guinea pig afferent vagal neurons to stimulation with hypertonic saline. Differences in sensitivity to activation were most obvious when the responses of jugular ganglion Aδ fibers were compared with those of nodose ganglion Aδ fibers. It was also observed that, within the population of jugular ganglion neurons investigated, the Aδ fibers were more responsive to challenge with hypertonic saline compared with the jugular ganglion C fibers. Unfortunately, the relative scarcity of C fibers projecting to the guinea pig trachea and main bronchi from the nodose ganglion precludes definitive conclusions as to the relative responsiveness of this fiber type, compared with either nodose ganglion Aδ fibers or fibers originating from the vagal jugular ganglion.

The mechanism by which hyperosmolar solutions stimulate afferent fibers is uncertain. It has been suggested that such mechanisms may involve changes in osmolarity per se or alterations in the concentration of permeant ions (28). Although a rigorous investigation of the mechanism by which hypertonic saline stimulates airway afferent fibers was not undertaken, it can be suggested that activation of mechanically sensitive jugular ganglion neurons by hypertonic saline may be dependent on the osmolarity of the solution rather than on the concentration of sodium and chloride ions, as the effect of the 4% NaCl-Krebs solution was mimicked by application of an equivosmolar solution of mannitol. This is in line with the findings of Garland and...
Inflammatory reactions in the airways. In the present study, inhalation of aerosols of hypertonic saline was found to cause significant plasma extravasation in the guinea pig trachea and main bronchi. That the response was blocked by a selective inhibitor of tachykinin NK₁ receptors indicated that it was likely to be secondary to stimulation of tachykinin-containing afferent nerve endings in the airway wall, although no simultaneous measurement of plasma extravasation and activation of vagal afferent fibers in vivo was made to confirm this point. The ability of aerosols of hypertonic saline to evoke neurogenic plasma extravasation in guinea pig airways is in agreement with studies in the rat (19, 27), where through selective vagotomy it has been shown that most of the neurons mediating neurogenic plasma extravasation have their cell bodies located in the jugular ganglion (18).

Reflexes stimulated specifically by jugular ganglion Aδ fibers in guinea pig airways are not known but may involve cough. Inhalation of hyperosmotic aerosols is a powerful stimulus for the induction of cough in human asthmatic subjects (1, 6, 22, 24) and causes cough in guinea pigs in vivo (15). The involvement of airway afferent fibers in the production of cough in humans and experimental animals has been recently reviewed (30), and it was suggested that the airway neurons responsible for the production of cough are small myelinated fibers, of which the principal candidate put forward was the rapidly adapting Aδ fibers. In the majority of patients with symptoms of asthma, inhalation of aerosols of hypertonic saline causes bronchoconstriction (1, 6, 22, 24). Significant airflow obstruction has also been reported after hypertonic saline aerosol challenge in the canine peripheral lung (10). Although the precise mechanism by which hypertonic saline produces airway narrowing remains unknown, data in the present study suggest that airway responses to hyperosmolarity may be the consequence of events resulting from activation of afferent airway C fibers and Aδ fibers arising predominantly from cell bodies in the jugular ganglion.

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Hypertonic saline stimulates jugular ganglion neurons


