Effect of bradykinin on membrane properties of guinea pig bronchial parasympathetic ganglion neurons

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Kajekar, Radhika, and Allen C. Myers. Effect of bradykinin on membrane properties of guinea pig bronchial parasympathetic ganglion neurons. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L485–L491, 2000.—The effect of bradykinin on membrane properties of parasympathetic ganglion neurons in isolated guinea pig bronchial tissue was studied using intracellular recording techniques. Bradykinin (1–100 nM) caused a reversible membrane potential depolarization of ganglion neurons that was not associated with a change in input resistance. The selective bradykinin B2 receptor antagonist HOE-140 inhibited bradykinin-induced membrane depolarizations. Furthermore, the cyclooxygenase inhibitor indomethacin attenuated bradykinin-induced membrane depolarizations to a similar magnitude (~70%) as HOE-140. However, neurokinin-1 and -3 receptor antagonists did not have similar inhibitory effects. The ability of bradykinin to directly alter active properties of parasympathetic ganglion neurons was also examined. Bradykinin (100 nM) significantly reduced the duration of the afterhyperpolarization (AHP) that followed four consecutive action potentials. The inhibitory effect of bradykinin on the AHP response was reversed by HOE-140 but not by indomethacin. These results indicate that bradykinin can stimulate airway parasympathetic ganglion neurons independent of sensory nerve activation and provide an alternative mechanism for regulating airway parasympathetic tone.

BRADYKININ AND RELATED kinins are produced endogenously in several tissues and are implicated in both physiological and pathophysiological conditions. In the airways, bradykinin exerts multiple effects, including bronchoconstriction, vasodilation, plasma extravasation, and cough (5, 26), by acting directly on effector cells or indirectly due to the release of other inflammatory mediators (10). In airway inflammatory diseases, such as allergic rhinitis and asthma, increased levels of circulating kinins have been detected (2, 30). The actions of bradykinin in the airways are proposed to be due, at least in part, to the stimulation of sensory afferent nerve endings leading to cholinergic and noncholinergic reflex activity (8, 15, 28).

Autonomic tone in the airway is under constant control by activity in preganglionic nerve fibers emanating from the central nervous system (CNS). Preganglionic activity from the CNS to the airways can be increased by stimulating afferent pathways that facilitate autonomic reflexes. This centrally mediated reflex is, indeed, one of the proposed mechanisms for bradykinin-induced bronchoconstriction in humans inasmuch as ipratropium bromide has been shown to attenuate the effects of bradykinin in vivo, but bradykinin has negligible effects on human bronchial smooth muscle in vitro (9), thus suggesting the requirement for an intact autonomic pathway to airway ganglia. Similarly, nasal hyperreactivity to bradykinin in allergic rhinitic subjects is attenuated following atropine treatment (27), providing further evidence that bradykinin initiates a central reflex.

In addition to central reflexes, parasympathetic nerve activity can be modulated locally by a peripheral reflex mechanism (21, 23). In a peripheral reflex, afferent nerve terminals (immunoreactive for neurokinins) project collateral branches directly to intrinsic parasympathetic ganglia (4, 6) and on activation lead to excitatory postsynaptic potentials in airway parasympathetic ganglion neurons independent of the CNS (18, 21). We have previously demonstrated that neurokinins released from capsaicin-sensitive afferent fibers can stimulate airway parasympathetic ganglion neurons through neurokinin receptors located on the ganglion neurons (21, 23). Although this evidence indicates that neurokinins may modulate airway cholinergic transmission, it is unclear whether this important regulatory response may have a role in airway inflammation and chemical irritation.

It is well recognized that bradykinin is a potent stimulant of sensory nerves in the airways (8, 14, 15). The purpose of this study was to evaluate whether bradykinin can 1) modulate parasympathetic nerve excitability in guinea pig airway by initiating a peripheral reflex, 2) indirectly affect bronchial parasympathetic nerves by releasing neuroactive mediators, and/or 3) directly stimulate bradykinin receptors on the parasympathetic ganglion neurons. Such effects may have important consequences in the regulation of parasympathetic tone in the inflamed airway.

METHODS

Tissue preparation. Male albino guinea pigs (Dunkin-Hartley) weighing 180–300 g were killed by a sharp blow to the head and exsanguinated. The thorax was opened, and the lungs, bronchi, and trachea were removed and placed in Krebs bicarbonate buffer (composition in mM: 118 NaCl, 5.4 KCl, 1.0 MgSO4, 1.9 CaCl2, 1.0 NaH2PO4, 25 NaHCO3, and 11.1...
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Dextrose (95% O2-5% CO2, pH 7.4).

The right bronchus was isolated from the trachea and lung parenchyma, cut along the ventral surface, and opened as a sheet. Ganglia were located without the aid of staining on the serosal surface of the right primary bronchus after removing the overlying connective tissue by fine dissection. The bronchus was transferred and pinned as a sheet to the sylgard lining of a recording chamber (0.05-mL volume) with volume minutes. Once in the recording chamber, the tissue was continuously superfused with Krebs bicarbonate buffer (36–37°C, 5–8 mL/min) and equilibrated for at least 60 min before experimentation.

Electrophysiological methods. Intracellular micropipettes were fabricated from thick-walled capillary stock (50-mM OD; World Precision Instruments; Sarasota, FL) by a Brown-Flaming microelectrode puller (model P-87, Sutter Instruments; San Rafael, CA). Electrodes were filled with an electrolyte solution of 3 M KCl (pH 7.4). The micropipettes were connected to an Ag-AgCl wire in an electrode holder (Axon Instruments; Foster City, CA) to an electrometer (Axodamp 2A, Axon Instruments), and an Ag-AgCl pellet in the bath was connected to the headstage ground. The electrode DC resistance in Krebs solution ranged between 50 and 60 MΩ. Impairment of the neurons was aided by a brief 20- to 50-ms overcompensation (i.e., buzz) of the capacitance neutralization circuit of the amplifier. Intracellular recordings were performed with the electrode in either discontinuous current clamp (3.0- to 4.0-kHz sampling rate) or active-bridge mode. The delivery of constant-current pulses through the microelectrode was controlled by a computer (Apple Macintosh, Cupertino, CA) equipped with a data-translation interface. Recorded intracellular data were displayed on-line with dual-beam oscilloscope and a chart recorder and stored on digital audiotape for off-line analysis. Data epochs were digitized and displayed using an oscilloscope-simulation and analysis program (AxoData and AxoGraph, Axon Instruments).

Baseline control membrane properties were made after the establishment of a stable recording [i.e., <1 mV change in resting potential and no change in input resistance (Ri)]. Changes in passive membrane properties, such as resting membrane potential and Ri, were constantly monitored following impairment of the neuron. Once a stable resting membrane potential was observed (usually 2–5 min after impairment), the Ri of the neuron was calculated from the steady-state amplitude of the voltage transient produced by a hyperpolarizing constant-current step (100 pA, 1- to 5-s duration). Changes in membrane resistance were also monitored continuously by noting changes in the amplitude of the electrotonic voltage transients produced by hyperpolarizing current steps (100 pA, 100–150 ms, 1 Hz). The effect of drug application on active membrane properties, including the duration and amplitude of the cumulative afterhyperpolarization (AHP) response that followed four consecutive action potentials (elicited by 3.0 nA, 20–33 Hz, 2-ms steps), were also measured. Cumulative AHP amplitude was measured from the prestimulus resting membrane potential to the peak AHP of the fourth spike; cumulative AHP duration was measured as the time from the point where the repolarization of the fourth spike equaled the prestimulus resting potential level up to the point when the AHP returned to the original resting potential. Control, cumulative AHP responses were monitored prior to drug application. Drug-induced changes in AHP responses were studied when the membrane potential returned to predrug levels but not more than 180 s after drug application. The accommodation characteristics of all neurons were also analyzed by noting the pattern of action potentials elicited during a series of incrementing depolarizing steps (500 ms, 1.0–2.0 nA). With this procedure neurons exhibit either continuous repetitive action potential discharge ("tonic" neurons) or an initial burst of action potentials that terminate within 100 ms of the onset of the depolarizing step ("phasic" neurons; Ref. 24).

Pharmacological responses. The effects of bath-applied bradykinin on active and passive membrane properties of guinea pig parasympathetic ganglion neurons were studied. Bradykinin, at doses (1–100 nM) previously shown to cause stimulation of tracheal and bronchial afferent nerve terminals and membrane depolarization of sensory ganglion neurons (14), was perfused directly over the parasympathetic ganglion preparation for 2–3 min, a period that allowed for a peak response to drug application to be observed. No more than two increasing concentrations were tested on any one ganglion neuron. A washout period of at least 10 min was used between increasing bradykinin concentrations. Bradykinin-induced changes in membrane potential, Ri, action potential accommodative properties, and cumulative AHP duration and amplitude were studied. The effects of bradykinin were also studied in the presence of the bradykinin B2 receptor antagonist HOE-140, the cyclooxygenase inhibitor indomethacin, and neurokinin-1 and -3 receptor antagonists SR-140333 and SR-142801, respectively. Neurokinin-1 and -3 receptor antagonists, ASM-substance P and senktide analog, respectively, were used in positive control studies. For antagonist studies, the tissue was pretreated for at least 10 min before the addition of bradykinin. Unless stated, for antagonist studies, bradykinin was tested once prior to antagonist pretreatment and then once after.

Materials. Bradykinin was obtained from Peninsula Laboratories (Belmont, CA). A stock solution of bradykinin (10 mM) was prepared in distilled water and stored in aliquots at −20°C. HOE-140 (d-Arg6-Hyp1,Trp5-Thr3,Pro2-Oic10-Val3-bradykinin) was a generous gift from Dr. K. Wirth (Hoechst; Frankfurt, Germany). Capsaicin and indomethacin were purchased from Sigma Chemical (St. Louis, MO). Stock solutions of capsaicin (50 mM) and indomethacin (10 mM) were prepared in 100% ethanol. SR-140333 and SR-142801 were generous gifts from Zeneca Pharmaceuticals (Wilmington, DE). SR-140333 was prepared as a stock solution (50 mM) in dimethyl sulfoxide. SR-142801 was prepared as a stock solution (10 mM) in distilled water. Acetyl-[Arg6,Sar9,Met(O2)11]SP6–11 (ASM-substance P), and [Asp5,Met9,Leu11]SP5–11 (sentide analog) were synthesized by Cambridge Research Biochemicals (Wilmington, DE). Final dilutions of all drugs were made in Krebs buffer solution. At the dilutions used in these studies dimethyl sulfoxide, ethanol, or distilled water had no measurable effect on the active or passive properties of parasympathetic ganglion neurons.

Data analysis. All data are expressed as the arithmetic means ± SE. Control values for resting membrane potential, Ri, cumulative AHP duration, and amplitude were noted prior to each drug application. These values were compared with peak changes evoked by drug application using one-way ANOVA followed by Student’s t-test. Statistical tests were performed using Statview statistics program (Abacus Concepts, Lafayette, CA). Statistical significance was accepted at the 0.05 level of probability.

RESULTS

Bath-applied bradykinin (1–100 nM) consistently depolarized the membrane potential of guinea pig bronchial parasympathetic ganglion neurons (Fig. 1A)
There was a threshold dose of 1 nM (n = 6; Fig. 1B). Control resting membrane potentials for bradykinin-induced effects at 1, 10, and 100 nM were −55 ± 3 mV (n = 6), −58 ± 4 mV (n = 6), and −56 ± 2 mV (n = 18), respectively. The lowest concentration tested (100 pM) had no effect on the resting potential (−54 ± 1 mV, n = 3). Bradykinin-induced changes in resting potential were concentration dependent (Fig. 1B). Responses for concentrations of bradykinin >100 nM were not studied, and, therefore, it was not possible to obtain accurate estimates of the EC50 values. Previous studies conducted in guinea pig vagal ganglia and tracheal and bronchial afferent nerve terminals indicate a similar effective dose range as displayed in Fig. 1B (14). During the peak of the depolarization response, there was no consistent change in Ri; in nine neurons, bradykinin (100 nM) caused a 40 ± 8% increase in Ri; in one neuron, there was a 50% decrease; and in five neurons, there was no change in Ri.

We postulated that the effects mediated by bradykinin in parasympathetic ganglion neurons are secondary to activating a peripheral reflex, i.e., stimulation of sensory nerve terminals with the consequent release of neuropeptides. We investigated this speculation by studying the effect of bradykinin in the presence of a combination of neurokinin-1 and -3 receptor antagonists SR-140333 and SR-142801 (1 µM), respectively. Pretreatment of the tissue with neurokinin-1 and -3 receptor antagonists did not significantly reverse the membrane depolarization response induced by bradykinin (100 nM; Fig. 2) but did, however, inhibit the depolarization induced by the sensory nerve stimulant capsaicin. The membrane depolarizations induced by the selective neurokinin-1 and -3 receptor agonists, ASM-substance P and senktide analog, respectively, were also blocked when studied in the presence of SR-140333 and SR-142801: control membrane potential = −66 ± 0.3 mV (n = 4) after pretreatment with SR-140333 (1 µM) and SR-142801 (1 µM); ASM-substance P (1 µM), 3 min = −65 mV (n = 2); senktide analog (50 nM), 3 min = −66 mV (n = 2; Ref. 23).

The membrane depolarization induced by bradykinin (100 nM) was significantly inhibited in the presence of the bradykinin B2 receptor antagonist HOE-140 (1 µM; Ref. 12; Fig. 3). In two of five neurons, bradykinin-induced depolarization in the presence of HOE-140 was associated with a 15 and 25% decrease in Ri, respectively, whereas the remaining neurons showed no change. The selective B1 agonist des-Arg10-kallidin (1 µM) and SR-140333 (1 µM) and SR-142801 (1 µM) are shown. Data are expressed as mean changes in membrane potential ± SE compared with predrug resting potential.
µM) failed to cause a significant change in membrane potential of guinea pig parasympathetic ganglion neurons: membrane potential at rest = 52 ± 6 mV; des-Arg10-kallidin (1 µM, 3 min) = 52 ± 6 mV (n = 2).

Pretreatment of the tissue with the cyclooxygenase inhibitor indomethacin (3 µM) significantly attenuated the membrane depolarization observed following bath application of bradykinin (100 nM; Fig. 4). This response was not associated with a significant change in R∞: indomethacin (3 µM) pretreatment = 41 ± 7 MΩ; bradykinin (100 nM) and indomethacin (3 µM) = 43 ± 6 MΩ (P > 0.05, n = 6).

Bradykinin (100 nM) decreased the duration of the cumulative AHP response (Fig. 5B). In contrast, indomethacin (3 µM) pretreatment did not significantly affect bradykinin-induced changes in the cumulative AHP response (Fig. 5B). In two neurons, the combined treatment of HOE-140 and indomethacin was studied. In these two neurons, indomethacin (3 µM) when administered alone inhibited the membrane depolarization response to bradykinin but failed to attenuate the decrease in duration of the cumulative AHP. After a 10-min “washout” period, these neurons were pretreated with a combination of indomethacin and HOE-140 (1 µM) and then reexposed to bradykinin (100 nM). The depolarization response to bradykinin was inhibited to the same level as when the tissue was pretreated with indomethacin alone, but the bradykinin-induced decrease in cumulative AHP duration was reversed to predrug levels [in 2 neurons, duration of AHP in the absence of drugs (control) = 175 and 171 ms; in the presence of bradykinin (100 nM) and indomethacin (3 µM), duration = 120 and 100 ms, respectively; in the presence of bradykinin, indomethacin and HOE-140, duration = 177 and 178 ms, respectively].

Bradykinin (1–100 nM) did not significantly alter the accommodative properties (tonic or phasic firing patterns) of bronchial parasympathetic ganglion neurons studied during a 500-ms suprathreshold stimulus. For example, 100 nM bradykinin did not change the accommodative properties of 11 neurons, five of which displayed a tonic action potential firing pattern and six neurons exhibited a phasic firing pattern (Fig. 6).

**DISCUSSION**

The results of the present study demonstrate the presence of functional bradykinin receptors on guinea pig primary bronchial tissue that have the capacity to modulate parasympathetic ganglion neuron excitability. We have previously provided data showing the presence of bradykinin receptors on sensory afferent
nerve terminals in guinea pig trachea and bronchus and on sensory vagal ganglion neurons (14). The latter results provided substantiating evidence for the ability of bradykinin to initiate a central sensory-parasympathetic reflex pathway in the airways. In the present study, superfusion of bradykinin onto intrinsic parasympathetic ganglia in guinea pig bronchus caused dose-dependent membrane depolarizations of the neurons. Similarly, stimulation of sensory nerves with the consequent release of neuropeptides, in particular substance P, has been shown to cause membrane depolarizations of parasympathetic ganglion neurons (21, 23). Thus it is conceivable that the stimulatory response to bradykinin is associated with a facilitation of the local release of proinflammatory neuropeptides. However, in the present study, the pretreatment of the tissue with neurokinin-1 and -3 receptors antagonists SR-140333 and SR-142801, respectively, did not alter the membrane depolarization induced by bradykinin but did block the membrane depolarization response to the sensory nerve stimulant capsaicin. We have shown previously that capsaicin-induced depolarization of membrane potential of bronchial ganglion neurons is sensitive to neurokinin-3 receptor antagonism (21). It is conceivable that higher concentrations of bradykinin are required to initiate a peripheral reflex or that neuropeptides other than neurokinins, e.g., calcitonin gene-related peptide, may be responsible for the depolarizing response to bradykinin. However, the concen-

![AHP response](image)

**Fig. 5.** Effect of bradykinin on AHP response evoked by 4 consecutive action potentials (3 nA, 2 ms, 20–33 Hz). A: representative intracellular recording trace showing duration and amplitude (shaded area) of cumulative AHP response in control and bradykinin (100 nM)-treated tissue. B: mean effect of bradykinin (100 nM) on cumulative AHP duration when administered alone (n = 6) and in presence of HOE-140 (1 µM, n = 5) and indomethacin (3 µM, n = 7). *P < 0.05. **P < 0.01.

![Action potential activity](image)

**Fig. 6.** Representative intracellular recording traces showing effect of bradykinin on action potential activity in phasic (A) and tonic (B) bronchial parasympathetic ganglion neurons. Neurons were subjected to suprathreshold current pulses (I; 1 nA, 500-ms duration) before (left) and 2–3 min after (right) superfusing the ganglion with bradykinin (100 nM). Bradykinin treatment did not alter firing pattern in phasic (n = 6) or tonic (n = 5) neurons. Scale bar is shown for both A and B.
tRATION OF BRADYKININ USED IN THIS STUDY CAUSED ROBUST Firing of afferent nerve terminals in guinea pig trachea and bronchus (8, 14), and, moreover, capsaicin-induced efferent release of neuropeptides and consequently the depolarization of parasympathetic ganglion neurons was sensitive to neurokinin receptor antagonism. Thus the bradykinin-induced membrane depolarization response observed in guinea pig bronchial parasympathetic ganglion neurons is unlikely to be exclusively due to the stimulation of sensory afferent fibers and consequent efferent release of neurokinins.

Consistent with findings in guinea pig (14) and rat (16) sensory vagal ganglia and rat superior cervical ganglia (1), bradykinin depolarizes guinea pig parasympathetic ganglion neurons via bradykinin B2 receptor activation. The B2 receptor antagonist HOE-140 markedly reduced bradykinin-induced membrane depolarization. Because the membrane depolarization response mediated by bradykinin does not appear to be secondary to sensory nerve stimulation (Fig. 2), we postulate that the bradykinin B2 receptors may be present on the parasympathetic ganglion neurons themselves and on activation are capable of directly modulating parasympathetic ganglion neuron excitability. Furthermore, bath application of the selective B2 receptor agonist des-Arg10-kallidin had no effect on the passive membrane properties of parasympathetic ganglion neurons. The B1 receptor is rarely expressed constitutively but is thought to be induced during inflammation (see Ref. 11 for review).

In contrast to studies in airway sensory afferent nerve terminals (8, 14) and in sympathetic ganglion neurons (1), bradykinin-induced depolarization of parasympathetic ganglion neurons was dependent on prostaglandin production. The cyclooxygenase inhibitor indomethacin inhibited (72%) the depolarization response to bradykinin by approximately the same magnitude as that seen with HOE-140 (75%). This suggests that the depolarization response induced by bradykinin is secondary to the production and/or release of prostaglandins and/or the activation of a common final pathway by these two groups of mediators. Several studies have demonstrated that the excitatory effects of bradykinin are dependent on the stimulation of the cyclooxygenase pathway of arachidonic acid metabolism and the resultant release of prostanoids (13, 17, 29). Bradykinin is known to stimulate the release of prostaglandins from several cell types (19, 25), including respiratory epithelial cells (3). On release, prostaglandins may result in sensitization or direct activation of neurons.

Bradykinin has been shown to increase the excitability of vagal sensory neurons by inhibiting a calcium-dependent, slow hyperpolarizing current (31, 32) observed after the falling phase of an action potential (AHP), and, moreover, this effect is indomethacin sensitive and can be mimicked by various prostaglandins (7, 33). In addition, in guinea pig bronchial parasympathetic ganglion neurons, the AHP response observed after cumulative action potentials has been demonstrated to be secondary to activation of a calcium-activated potassium current (22). In the present study we report an inhibition by bradykinin of the duration of the AHP induced by cumulative action potentials. This inhibitory effect is mediated via bradykinin B2 receptors, but in contrast to the studies in guinea pig sensory ganglia (33), this response is not secondary to the production of prostaglandins. Thus in guinea pig parasympathetic ganglion neurons, whereas the depolarization response induced by bradykinin is dependent on the release of prostaglandins, the inhibition of the cumulative AHP duration is not. That both responses can be inhibited by HOE-140, whereas only the depolarization response is attenuated in the presence of indomethacin, suggests that the decrease in cumulative AHP duration is mediated by bradykinin B2 receptors that may have direct effects on the active properties of parasympathetic ganglion neurons.

The present study provides substantiating evidence that the effect of bradykinin on guinea pig parasympathetic neurons may be a direct effect on receptors located on ganglion neurons. The ability of bradykinin to inhibit the duration of the cumulative AHP response may result in a decrease in the filtering capacity of these parasympathetic ganglion neurons (20, 22, 24). This may have important consequences during high-frequency presynaptic stimuli because it is likely that parasympathetic ganglionic output will be enhanced. It has been demonstrated previously that airway parasympathetic ganglion neurons have the capacity to regulate preganglionic input from the CNS in vivo (20) and in vitro (24).

Bradykinin has been associated with many inflammatory responses in the airways. Bradykinin is a potent stimulant of sensory afferent fibers in guinea pig airways (14), which may be important in evoking central-parasympathetic reflex activity during inflammation. The data provided here demonstrate that in addition to increasing parasympathetic preganglionic drive in the airways via a central reflex, this enhanced output may be further amplified by altering the excitability of airway parasympathetic ganglion neurons and thus the efficacy of neuronal output from airway ganglia and consequently local cholinergic control of airway function.

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